Diet–gene interactions: Characterisation of risk

Report of a workshop held on 23–24 February 2000

MRC Institute for Environment and Health

FORA 3

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Preface

This report is based on a workshop which was convened as part of the Food Standards Agency-sponsored Food Risk Assessment (FORA) project — a collaborative research programme between the MRC Toxicology Unit and the MRC Institute for Environment and Health in Leicester and the MRC Dunn Human Nutrition Unit in Cambridge. The programme aims to consider and conduct research into fundamental and applied approaches to risk assessment, most specifically in the context of diet and cancer.

Workshop participants are listed at the end of the report. Many thanks are due to all the workshop participants, and especially to the speakers and discussions leaders for their valuable contributions.

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Executive summary

Genetic and environmental factors may contribute to the aetiology of apparently sporadic cancers, and recent molecular cancer epidemiology studies have provided evidence suggesting that gene–environment interactions may impact on the progression of some cancers.

In this context, the influence of diet on cancer and on colorectal cancer in particular has long been recognised. Furthermore, there is an increasing understanding of genetic influences on cancer, again particularly on colorectal cancer. Taken together with developing technologies, especially in the fields of genomics and proteomics, which can contribute to increased understanding of gene expression, it has been timely to consider the impact of diet–gene interactions on the aetiology of cancer, and to assess how the new methodologies may provide new ways to help characterise cancer risks associated with diet–gene interactions.

In this report, based on a workshop convened as part of a project sponsored by the Food Standards Agency on Food Risk Assessment (FORA), discussions are presented on the characterisation of risks associated with diet–gene interactions. The report considers, in particular, the impact of such interactions on colorectal cancer and, to some extent, their impact on pancreatic cancer, for which there is also evidence for environmental and genetic components in the aetiology of the disease.

The aim of the workshop was to bring together experts in molecular epidemiology, cancer, genomics and proteomics to seek ways, including new research, to move towards a better understanding of the impact on health of diet–gene interactions.

The report of the workshop reaches the following conclusions.

- There is clearly a need to improve understanding about diet–gene interactions as, although current knowledge points to some intriguing indications, there are as yet no examples of clearly identified and characterised interactions. It is important to understand better the effect of diet on genes as well as the effect of genes on diet.

- The further development, in particular to improve cost and through-put, of several new methodologies, such as microarray techniques, proteomics and short oligonucleotide mass analysis, will facilitate studies on gene function and also on gene linkage. This will help identify not only genetic factors that predispose to disease or to a particular response to dietary components but also changes in gene expression that are part of the response to a dietary exposure.

- Several biomarkers of exposure are already available for use in molecular epidemiology studies. There is a need to develop intermediate biomarkers of risk, for example mutations in key genes, on the causal pathway from exposure to outcome.

- Epidemiological studies should be well designed and more effective methodologies for dietary analysis should be developed. Further research effort should be invested in studies that are well supported clinically, with appropriate identification of exposure and clinical characteristics and properly conducted statistical analyses.

A number of possible future research directions are proposed.

- Studies in migrant populations and ethnic groups within UK, to look at patterns of health, cancer incidence, dietary exposure and genetic susceptibility, should be undertaken, maximising

the opportunity presented by the presence of sizeable migrant and ethnic groups in certain regions in the UK. Studies in such UK populations may be particularly informative as some groups now comprise two or three generations, and variations between generations (especially in migrant populations) could be investigated. Studies in migrant populations could also investigate how much age at migration influences disease outcome.

- Epidemiological studies on diet and health could investigate populations of different ages, rather than just concentrating, as has been the case until now, on prospective studies in middle age.

- Future epidemiological studies should record the most detailed measures of dietary intake as are possible and practical. The development of better food composition tables is encouraged in order to facilitate the accurate production of such dietary records.

- Subject to appropriate ethical control, family groups of hereditary pancreatitis patients would provide well-motivated subjects to investigate how diet may affect disease outcome in high-risk groups.

- Mutation profiles in specific genes in humans could be used to study the aetiology of disease across different population (including migrant) subgroups. Mutation profiles could also be used to investigate temporal differences in response (i.e. how profiles vary according to the stage of life at which critical lesions occur), to see if specific mutations can be identified following specific exposures, and to investigate differences in 'background' profiles. The possibility of screening serum for DNA mutations to identify 'at risk' individuals could be investigated.

- Experimental studies could investigate both the relative contributions of diet and polymorphisms in the level of gene expression and activity of, for example, xenobiotic metabolising enzymes and the extent to which food components can up- or down-regulate protective mechanisms.

1 General introduction

1.1 Background

Factors such as genetics, environment and the play of chance may contribute in some way to the aetiology of sporadic cancers. A recent and influential report on nutritional aspects of the development of cancer (Department of Health, 1998) stated that:

> It is impossible, at present, to disentangle the relative contributions that each of these factors makes to the risk of cancer in an individual or within a given population.

However, recent results in molecular cancer epidemiology suggest that, for example, there is evidence for a gene–environment interaction in the progression of colorectal cancer, a disease for which dietary factors are thought to play an important role (Ulrich *et al.,* 1999). Furthermore, the rapidly accelerating pace of progress in the fields of genomics and proteomics over the past five years means that new technologies are available to examine the up- or down-regulation and misfunction of genes and their products in ways that involve minimal, if any, initial assumptions about the pathways involved.

To identify research strategies and opportunities which would make best use of new knowledge and technology in the characterisation of cancer risk from diet–gene interactions, the MRC Institute for Environment and Health (IEH) hosted a workshop held in Leicester in February 2000. This workshop was part of the Food Standards Agency-sponsored Food Risk Assessment (FORA) project, which is a collaborative research programme between the MRC Toxicology Unit and IEH in Leicester and the MRC Dunn Human Nutrition Unit in Cambridge.

The aim of the workshop was to bring together scientists with expertise in the fields of molecular epidemiology of cancer, genomics and proteomics in order to examine factors of importance in characterising cancer risk from diet–gene interactions and the contribution that might be made by the new technologies. Participants addressed four main topics:

- what is known about diet–gene interactions and how current knowledge contributes to characterising dietary risk factors;
- new technologies in genomics and proteomics, including mass spectrometry, that could be developed to investigate diet–gene interactions;
- the integration of epidemiology and toxicology, and the role of biomarkers in characterising diet–gene interactions; and
- approaches (based on the above) to improving the characterisation of the risks resulting from diet–gene interaction.

Short summary papers prepared for the workshop by invited speakers are presented in Sections 1.2 and 2.1 to 2.4; these were pre-circulated to workshop participants and provided the basis for discussions at the workshop. Current knowledge about diet–gene interactions in chronic diseases such as cardiovascular disease, osteoporosis and cancer are summarised in Section 1.2. Molecular epidemiological studies of the influences of diet and genetics on the risk of colorectal and pancreatic cancer are summarised in Sections 2.1 and 2.2. Some principles of genomics and proteomics are outlined in Sections 2.3 and 2.4 along with a consideration of the kinds of information that they can provide on changes in gene expression in response to environmental or other factors. Section 2.5 was prepared subsequent to the workshop in the light of discussions on general principles pertaining to the identification of different disease phenotypes within a population.

Finally, a summary of the discussions at the workshop is given in Section 3, and the conclusions reached during the discussions and recommendations for the future are presented in Section 4.

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1.2 Diet–gene interactions in chronic disease such as cancer

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There is a substantial body of evidence to show that the large international differences in the occurrence of most chronic diseases of middle and later life such as cancer are mainly due to environmental factors, such as diet. However, the influence of dietary factors on the relative risks of disease *within* populations is generally found to be small, perhaps because of the large measurement errors associated with dietary assessments, which are often used in epidemiological studies. In addition, dietary effects may be modulated by inherited polymorphisms in a number of genes encoding products involved in nutrient and xenobiotic metabolism, such as lipoproteins and phase I and phase II enzymes (Idle, 1991; Vineis, 1997). Some genotypic variants in combination with certain dietary patterns may greatly enhance cancer risk (Lang *et al*., 1994; Roberts-Thompson *et al.,* 1996). This interaction between nutritional and genetic factors (and with other environmental factors such as exercise, social factors or infection) has so far largely gone uninvestigated. The expected rapid progress in technology over the next few years will transform genetic profiling of individuals, and epidemiological trials which have collected both genetic material and data on environmental exposure will be at the forefront of studies aimed at strengthening understanding of diet–disease relationships. To date, however, there is only a limited amount of evidence of diet–gene interactions in cancer and other chronic disease such as heart disease. One recently well-publicised trial of the interaction between the different alleles of the acetylcholinesterase (ACE) gene and muscle performance after aerobic training is a good example of the interactions that can occur between genes and environmental factors (Williams *et al.,* 2000).

Gene–nutrient interactions in cancer

There are a number of polymorphisms in genes controlling the metabolism of dietary items, hormonal factors and xenobiotics that may be candidates for an association with an increased risk of cancer. These genes include those encoding glutathione-*S*-transferases M1, M3, T1 (GSTM1, GSTM3, GSTT1), *N*-acetyltransferases 1 and 2 (NAT1, NAT2), apolipoprotein E (ApoE), vitamin D receptor (VDR) and two members of the cytochrome P450 family (CYP1A1 and CYP2D6). For example, the heterocyclic amines present in cooked meat require NAT for activation as carcinogens and different ApoE phenotypes are associated with altered risks of colorectal cancer and cardiovascular disease (CVD) (Kervinen *et al.,* 1996). Vitamin D is involved in the regulation of cell proliferation and differentiation, and the receptor is present in a variety of normal tissues and organs, tumour tissues and cancer cell lines. The presence of variants of the receptor gene has been correlated with altered circulating levels of active vitamin D hormone, gene expression and cancer risk (Taylor *et al.,* 1996). Methods for estimating methylenetetrahydrofolate reductase (MTHFR) have also been introduced. This enzyme catalyses the synthesis of 5-methyltetrahydrofolate, a methyl donor involved in the conversion of homocysteine to methionine, from 5,10-methylene-tetrahydrofolate, and is one of the enzymes responsible for methyl supply, DNA methylation and thymidine formation (Ma *et al.,* 1997). Genetic variants of MTHFR would be expected to disrupt a number of metabolic pathways and hence alter disease risk. In oral cancer the association with alcohol is considerably strengthened when polymorphisms in alcohol dehydrogenase (ADH) type 3 are taken into account (Harty *et al.,* 1997). In heavy drinkers, relative risks for oral cancer (relative to non-drinkers) increase to 40.1 (confidence interval 5.4–296) for ADH3 1-1 allele individuals (fast alcohol to acetaldehyde metabolisers) compared with 4.4 for ADH3 2-2 individuals (Harty *et al.,* 1997).

Possible gene–nutrient interactions in cancer are discussed further by other contributors in Section 2. Here examples will be given from other chronic conditions.

Gene–nutrient interactions in mammographic patterns in relation to phytoestrogen intake

A dense mammographic pattern is a risk factor for breast cancer which is modifiable by the antioestrogen tamoxifen (Atkinson *et al.,* 1999). Polymorphisms in genes controlling steroid metabolism are thought to modulate breast and prostate cancer risk, and to determine interactions between hormonal, phytoestrogen, and lipid responses. They include polymorphisms in the genes encoding the oestrogen receptors α and β, CYP17 (17 α hydroxylase), controlling the conversion of progesterone to androstenedione, and CYP19 (P450 aromatase), controlling the conversion of androstenedione to oestrone (Gharani *et al.,* 1997). However, ongoing studies

have not revealed significant interactions between polymorphisms in those genes and response to phytoestrogens on mammographic density, possibly because only small sample sizes have been available as yet (Atkinson, 2000).

Gene–nutrient interactions in cardiovascular disease

Numerous epidemiological studies have demonstrated a strong and consistent relationship between serum cholesterol levels and risk of coronary heart disease (CHD). However, the within-population relationships between diet and serum cholesterol levels are not strong, partly because of interaction with genes controlling lipid metabolism. Dietary responses have been shown to vary according to ApoE genotype, with cholesterol absorption being enhanced in those individuals with an E4 allele which encodes an apoprotein that binds preferentially to very low density lipoprotein (VLDL). Individuals with the ApoE4 allele show a greater increase in serum cholesterol than those with the E2/E3 alleles in response to a high saturated fat diet (Sarkkinen *et al.,* 1998). This effect has not been shown in published epidemiological studies, but is evident from studies within a small sample of the European Prospective Investigation of Cancer (EPIC). In this sample, the association between dietary saturated fat and serum LDL cholesterol was small (correlation coefficient, 0.2) in the population as a whole but much greater (0.5) in those individuals with the ApoE4 allele (Loktionov *et al.,* 2000).

There are also clotting factor associated genotypes which may demonstrate interrelation with dietary factors. An increased circulating level of fibrinogen is a well-established risk factor for CVD. A common G/A sequence variation in the β-fibrinogen gene has been found at nucleotide position 455 and there is some evidence that there may be differential effects on clotting according to environment (Humphries *et al.,* 1997). In addition, certain polymorphisms of the single gene (on chromosome 13) encoding circulating factor VII are also associated with increased risk of CVD. A common G to A polymorphism mapping at codon 353 of exon 8 modulates the level of factor VII and plasma triglycerides (Wu, 1997). Two studies have investigated the effect of a diet containing fat on individuals in whom the R353Q polymorphism has been identified. In the first, a high fat diet increased the active form of factor VII in males irrespective of genotype (Sanders *et al.,*1999). In the second, in elderly women, there was a greater increase in active factor VII in RR homozygotes compared with those carrying the Q allele (Mennen *et al.,* 1999).

The plasminogen activator–inhibitor type 1 (PAI-1) phenotype also influences the risk of CHD and there is a common polymorphism in the promoter region of PAI-1 lying 675 base pairs upstream from the start of transcription where either four or five Gs are present in about equal frequency: the 5G allele encodes an additional protein binding site and the 4G allele is associated with increased PAI-1 levels and a greater overall risk of CHD (Humphries *et al.,* 1997). However, the interaction with dietary factors important in clotting and these genotypes has not been assessed, although there are published examples of the interaction between genotype and other environmental variables in clotting (Humphries *et al.,* 1997).

Other routes by which diet might affect risk of CHD involve paraoxonase. The precursor lesion of the atheroma is the fatty streak, which is formed when macrophages take up LDL which has been modified by oxidation or glycosylation via the acetyl LDL receptor to form 'foam' cells. Paraoxonase has been shown to prevent oxidation of LDL *in vivo*, and the 10- to 40-fold variation in LDL levels seen with different paraoxonase phenotypes may be related to CHD risk. The gene encoding paraoxonase (*HUMPONA*) is located on chromosome 7q21–22 and codes for a protein of 355 amino acids. A glutamine/arginine polymorphism at amino acid position 192 determines the activity of the enzyme, and levels of paraoxonase and CHD risk (Marian, 1997). However, interactions with dietary antioxidants (vitamins E, C and the flavonoids and carotenes) have not yet been investigated.

Gene–nutrient interactions in osteoporosis

A number of dietary items are thought to be required to reduce the risk of osteoporosis and maintain bone strength, including calcium, phosphorus, sodium, vitamin D and vitamin K, required for carboxylation of osteocalcin. The phytoestrogens, which bind to β-oestrogen receptors in bone (Kuiper *et al.,* 1997; Ralston, 1997), may also influence the process. In addition, there are several candidate gene polymorphisms which may be involved in the process, such as the VDR gene and the collagen type 1 alpha gene (Ralston, 1997). To date, it has not been possible to show differential effects of phytoestrogens on bone density in relation to VDR gene polymorphisms (Atkinson, 2000).

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2 Assessing diet–gene and environment–gene interactions

2.1 Molecular epidemiology and colorectal cancer

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Introduction

Colorectal cancer is a major cause of death in many western countries. Up to 5% of cases are due to inherited forms of the disease, in particular familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC), but the aetiology of the sporadic form of the disease has proved more difficult to determine. However, some evidence for increased relative risk for certain dietary, environmental and genetic factors has been reported (Potter *et al.,* 1993). Of these factors, diet has been the most widely studied. Red meat intake has been shown to be associated with increased risk of the disease in a number of different studies, including case–control, cohort and migration (Haesnzel *et al.,* 1973; Armstrong & Doll, 1975; Willett *et al.,* 1990). In addition, method and extent of cooking may affect risk of the disease, with intake of well-cooked, barbecued meat associated with the highest risk (De Verdier *et al.,* 1991). An association between alcohol consumption and susceptibility to rectal cancer has also been reported (Longnecker, 1990).

The increased risk of development of colorectal cancer associated with meat eating may reflect an important role for heterocyclic amines as chemical carcinogens in this disease. Heterocyclic amines are formed as pyrolysis products during cooking and have been demonstrated to be both mutagenic and carcinogenic (Gross *et al.,* 1993). High concentrations of heterocyclic amines such as MeIQX and PhiP have been demonstrated in grilled meat and fish (Skog *et al.,* 1995).

It has been reported that polymorphisms in genes encoding xenobiotic activating and metabolising enzymes, particularly the cytochrome P450 CYP1A2, the *N*-acetyltransferases and the glutathione *S*-transferases, modulate risk of colorectal cancer development (see below). This relationship may be due to the role of these enzymes in heterocyclic amine activation and detoxification. It is known that heterocyclic amines undergo activation to mutagens in a series of reactions initiated by *N*-oxidation by cytochromes P450, particularly CYP1A2 (Turesky *et al.,* 1991). The oxidised metabolite may undergo *O*-acetylation by hepatic *N*-acetyltransferases directly or may be glucuronidated and excreted in the bile followed by cleavage of the glucuronic acid moiety and *O*-acetylation in the colon (Minchin *et al.,* 1992). A nitrenium ion, formed from the *O*-acetylated compound, is believed to be the ultimate mutagen. Glutathione *S*-transferases appear to detoxicate the *O*-acetylated compounds but the precise isoforms involved in this reaction in humans are still not clear (Lin *et al.,* 1994).

Cytochrome P450 polymorphisms

CYP1A2 has been the P450 isoform studied most extensively in relation to risk of colorectal cancer, mainly because it is the enzyme that oxidises dietary heterocyclic amines with the highest efficiency (Butler *et al.,* 1989). Expression of CYP1A2 occurs at high levels only in the liver and is inducible by various environmental agents, including tobacco smoke and charcoal-grilled food, probably through the aryl hydrocarbon receptor (Swanson & Bradfield, 1993). CYP1A2 levels vary widely between individuals and, on the basis of phenotyping studies with caffeine, it has been suggested that a genetic polymorphism occurs in

CYP1A2 which results in individuals being either fast, intermediate or slow metabolisers (Butler *et al.,* 1992). A study on colorectal cancer susceptibility suggested that individuals with a fast metaboliser CYP1A2 phenotype and a high consumption of red meat were at significantly increased risk of developing the disease (Lang *et al.,* 1994). However, confirming the existence of a phenotypic polymorphism in CYP1A2 and the genetic basis of such a polymorphism has been problematic. A recent study of caffeine phenotypes in healthy non-smoking British Caucasians found that, while individuals showed approximately 20-fold variation in the caffeine metabolic ratio, believed to be a measure of CYP1A2 activity (1,7-dimethyluric acid and 1,7-dimethylxanthine/1,3,7-trimethylxanthine; (17U + 17X/137X), the data could be fitted to a log-normal distribution rather than the bimodal or trimodal distribution expected where genetic polymorphism occurs (Welfare *et al.,* 1999a). Also, analysis of the coding sequence and some upstream sequence failed to detect any functionally significant polymorphisms (Welfare *et al.,* 1999a). However, a polymorphism in intron 1 of CYP1A2 which appears to relate to CYP1A2 activity in smokers has recently been detected in Caucasians (Sachse *et al.,* 1999), as have several upstream region polymorphisms in Japanese populations (Nakajima *et al.,* 1999). A polymorphism resulting in an amino acid substitution has also been detected in a Chinese subject but was found to be very rare (Huang *et al.,* 1999).

A polymorphism in the aryl hydrocarbon receptor has recently been found apparently to cosegregate with high induced CYP1A1 activity (Smart & Daly, 2000) and could also be relevant to CYP1A2 induction. Benzo[a]pyrene may be present in barbecued food, and polymorphism in the gene encoding its main activating enzyme, CYP1A1, might also be relevant to colorectal cancer risk but, as for CYP1A2, the existence of functionally significant polymorphisms in CYP1A1 remains controversial (Smart & Daly, 2000).

N-acetyltransferase polymorphisms

On present evidence, polymorphism in *N*-acetyltransferase 2 (NAT2) is the best candidate for a metabolic polymorphism likely to modulate risk of colorectal cancer development because of the relevance of substrates such as dietary heterocyclic amines to colorectal cancer. Several different base changes can result in loss of enzymic activity (Grant *et al.,* 1997) and the deficient (slow acetylator) phenotype is seen in between 10 and 90% of individuals, depending on ethnic origin (Evans, 1989). Approximately 50% of Caucasian are slow acetylators. The other human *N*-acetyltransferase, NAT1, has also now been demonstrated to exhibit polymorphism, but variant alleles associated with absence of activity are rare and therefore of less importance in assessing overall risks in populations (Butcher *et al.,* 1998). A common allele (*NAT1*10*), suggested to be associated with faster than normal metabolism, has also been described but its phenotypic effect has not been confirmed in all studies (Bell *et al.,* 1995; Hughes *et al.,* 1998).

Early studies on NAT2 polymorphism in relation to colorectal cancer susceptibility used phenotyping with a probe drug to determine metabolic phenotype, and a meta-analysis of all data of this type suggests a positive association between colorectal cancer susceptibility and the fast acetylator phenotype (d'Errico *et al.,* 1999). More recent studies have generally used genotyping, which correctly identifies acetylator status in over 95% of subjects, and these studies show no overall association between genotype and susceptibility (Table 2.1). However, several studies have also examined dietary factors in addition to genotype and phenotype. In general these studies have tended to show a relationship between the rapid NAT2 genotype and susceptibility to colorectal cancer mainly in individuals with a high meat consumption with cooking method also important (Roberts-Thompson *et al.,* 1996; Welfare *et al.,* 1997; Chen *et al.,* 1998; see Table 2.1). There is also some evidence that being positive for the *NAT1*10* allele further increases risk in these individuals (Bell *et al.,* 1995; Chen *et al.,* 1998).

In addition to a positive association between the rapid acetylation phenotype and high levels of meat consumption, one study has found an increased incidence of the disease among slow acetylators who smoke or have a high alcohol consumption (Welfare *et al.,* 1997).

Glutathione *S*-transferase polymorphisms

Several members of the glutathione *S*-transferase (GST) superfamily show polymorphism, with complete loss of function polymorphisms occurring in GSTM1 and GSTT1, while in the case of GSTP1 two alleles which give rise to amino acid substitutions in the gene product have been described. One of these amino acid substitutions appears to be associated with altered kinetics with several substrates, but these effects are relatively small. The relationship between each of these GST polymorphisms and colorectal cancer susceptibility has been studied. Most studies have not detected

Table 2.1 Previous studies on NAT2 genotype/phenotype in relation to colorectal cancer susceptibility

Author	No. of cases	No. of controls	Method	Odds ratio for disease development in fast acetylators (95% CI)	Odds ratio from subgroup analysis (95% CI)
Lang *et al.* (1986)	43	41	P	2.48 (1.02–6.03)	
Ilett *et al.* (1987)	49	41	P	2.41 (1.18–4.95)	
Ladero *et al.* (1991)	109	96	P	1.14 (0.66–1.99)	
Rodriguez *et al.* (1993)	44	28	G	0.96 (0.37–2.49)	
Shibuta *et al.* (1994)	234	329	G	0.83 (0.48–1.57)	
Oda *et al.* (1994)	36	36	G	1.00 (0.19–5.32)	
Lang *et al.* (1994)	34	205	P	0.86 (0.39–1.90)	2.79 (1.69–4.47) (NAT2 rapid/CYP1A2 rapid)
Spurr *et al.* (1995)	103	96	G	1.22 (0.65–2.29)	
Bell *et al.* (1995)	202	112	G	1.1 (0.71–1.8)	2.0 (1.1–3.8) (NAT2 rapid, *NAT1*10* positive)
Roberts-Thompson *et al.* (1996)	61	110	G/P	1.8 (1.0–13.3)	
Welfare *et al.* (1997)	174	174	G	0.95 (0.61–1.49)	6.04 (1.6–26) (NAT2 rapid/frequent fried meat)
Chen *et al.* (1998)	212	221	G	0.80 (0.53–1.91)	5.82 (1.11–30.6) (NAT2 rapid/*NAT1*10* positive/high meat consumption)
Gil & Lechner (1998)	114	201	G	1.62 (0.97–2.68)	4.23 (2.02–8.86) (*NAT2*4/*4* genotype)

95% CI, 95% confidence interval; G, genotyping analysis; P, phenotyping analysis

positive associations apart from one study suggesting that GSTM1 null individuals were at increased risk of proximal colon cancer (Zhong *et al.,* 1993), and one suggesting that GSTT1 null subjects had an increased risk of colorectal cancer generally (Deakin *et al.,* 1996). A recent study looking at combined NAT2/GSTT1 genotypes detected a significant association with colorectal cancer susceptibility in individuals with a slow NAT2 and null GSTT1 genotype (Welfare *et al.,* 1999b). However, the possibility that this is a chance association cannot be excluded. No associations with any GSTP1 genotypes have been detected. Consideration of dietary factors such as red meat consumption did not alter the overall negative findings of a lack of association between GST genotypes and colorectal cancer susceptibility (Welfare *et al.*, 1999b).

Methylenetetrahydrofolate reductase polymorphism

A polymorphism resulting in decreased activity due to an amino acid substitution has recently been detected in the methylenetetrahydrofolate reductase (MTHFR) gene. MTHFR plays an important role in folate metabolism, with its product 5-methyltetrahydrofolate being the main form of folate in plasma, whereas the substrate methylenetetrahydrofolate is mainly found within cells. Methyltetrahydrofolate provides the methyl groups for methionine synthesis from homocysteine and for DNA methylation. Hypomethylation of DNA is a common feature of colonic neoplasms (Laird & Jaenisch, 1994). However, methylenetetrahydrofolate also has a specific role in the conversion of deoxyuridylate to thymidylate and its deficiency could lead to DNA strand breaks. Low-folate diets and alcohol consumption, which decreases folate absorption, may affect levels of both

methyl- and methylenetetrahydrofolate, but precise effects may vary depending on levels of MTHFR.

A diet low in folate is a known risk factor for colon cancer and risks are increased when this is combined with high alcohol consumption. In a study on the relationship between MTHFR polymorphisms and colorectal cancer based on the US Physicians' Health Study (Ma *et al.,* 1997), it was found that men with two copies of the low-activity MTHFR allele had a decreased risk of cancer development, with the strongest protection observed in those with high plasma folate levels. However, no protection was observed in those with low folate levels. A similar effect was observed for alcohol intake, with those consuming more than 1 unit of alcohol per day losing the protection from low-activity MTHFR alleles and in fact becoming the genotype group with the highest risk of colorectal cancer. Another US-based case–control study has obtained broadly similar data (Slattery *et al.,* 1999).

We have recently examined the relationship between MTHFR alleles and colorectal cancer among cases and controls from north east England studied previously for other genetic polymorphisms (Welfare *et al.,* 1997). However, unlike previous studies on MTHFR, we found that possession of two variant MTHFR alleles resulted in an increased risk of disease (Table 2.2; Welfare M & Daly AK, unpublished). Alcohol consumption in the subjects in our study was higher than in the US study (mean 2.5 units per day for men compared with 0.6 units per day) but it was not possible to compare plasma folate levels. These findings may suggest that the effect of the MTHFR polymorphism on colorectal cancer susceptibility is modulated by both dietary folate and alcohol, with high MTHFR being a risk factor when folate levels are high and a protective factor when they are low.

Other polymorphisms

Phase II xenobiotic metabolising enzymes, including both UDP-glucuronosyltransferases and sulfotransferases, have a major role in the metabolism of dietary heterocyclic amines implicated in colorectal cancer. Sulfotransferases may catalyse activation of heterocyclic amines (Chou *et al.,* 1995) and UDP-glucuronosyltransferases have a role in both *O*-glucuronidation of heterocyclic arylamines already oxidised by P450 in the liver (Bock, 1992) and in the *N*-glucuronidation of the amino group (Stillwell *et al.,* 1999). There is increasing evidence that functionally significant polymorphisms occur in both the UDP-glucuronosyltransferases and sulfotransferases, but studies on their association with colorectal cancer susceptibility do not yet appear to have been reported.

Table 2.2 Methylenetetrahydrofolate reductase genotypes in colorectal cancer cases and controls from north east England

Genotype	Cases (n = 195)	Controls (n = 177)
T667/T667	32 (0.16)	15 (0.085)
T667/C667 or C667/C667	163 (0.84)	162 (0.92)

Odds ratio for the homozygous T677 genotype: 2.12 (95% CI 1.10–4.06; p = 0.028)

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2.2 Molecular epidemiology and pancreatic cancer

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Introduction

Perhaps the best example of an important diet–gene interaction comes from a serious non-malignant disorder: phenylketonuria (PKU). This well-characterised disease is caused by an inherited genetic defect, which can be detected at birth by an inexpensive, widely used blood test. For neonates testing positive, dietary modification will prevent the mental deterioration that inevitably develops in untreated infants. The aim of studies of diet–gene interactions in various malignant diseases is to identify similarly effective strategies to lower the risk of major cancers. Because little is known about diet–gene interactions in breast and prostate cancer, this survey focuses on pancreatic cancer.

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Diet and pancreatic cancer

About 25–35% of all pancreatic cancers have been attributed to diet. These estimates are rather imprecise, but suggest that detection of any diet–gene interaction could have important implications for prevention of this lethal cancer. As with several other tumours, the risk of cancer is thought to be increased by excess caloric intake, high fat diets, cured meat, and diminished intake of fruit and vegetables. Heterocyclic amines, which are present in cooked foods, might be the link between diet and pancreatic cancer. Putative aromatic amine–DNA adducts have been detected in human pancreatic tissue (Anderson *et al.*, 1997).

Polymorphic genes

It seems reasonable that genetic variation in enzymes responsible for degradation of dietary carcinogens could increase or decrease the risk of pancreatic cancer. Table 2.3 lists reports in both animals and humans concerning several polymorphic genes that have been studied in relation to the pancreas. Within the pancreas,

Table 2.3 Polymorphic genes and pancreatic disease

Reference	Human or animal	Tissue type	Findings
Foster *et al.* (1993)	Human	Organ donors, CP, PACA	Increased cytochrome P450 levels in CP or PACA compared with normal tissue
Johnson *et al.* (1994)	Human	Normal pancreas	VDR present in normal human pancreas, especially islet cells
Bartsch *et al.* (1998)	Human	Blood from CP, PACA, and control subjects	Non-significant excess of NAT1 slow acetylators in cases compared with controls. Over-representation of GSTM1 AB or B genotype in CP and PACA
Andersen *et al.* (1997)	Human	Organ donors	NAT1 is the predominant *N*-acetyltransferase in pancreas. "Aromatic amines and nitroaromatic hydrocarbons may be involved in pancreatic cancer."
Wacke *et al.* (1998)	Human	CP Patients, normal pancreas	Up-regulation of cytochrome P450 in CP compared with normal tissue
Thompson *et al.* (1999)	Human	Normal pancreas	Slow NAT1*4/*4 genotype may be associated with detoxification of 4–aminobiphenyl
Lin *et al.* (1986)	Baboon	Normal pancreas	Apolipoprotein E activity found in normal baboon pancreas
Mori *et al.* (1995)	Hamster	Pancreatic tissue after animal exposed to smoke	Cigarette smoke might stimulate metabolic activation of food derived carcinogens.
Kessova *et al.* (1998)	Rat	Normal pancreas	Ethanol and methylcholanthrene exert striking inductive effects on cytochromes P450 2E1 and P450 1A1

CP, chronic pancreatitis; GST, glutathione-*S*-transferase; NAT, *N*-acetyltransferase;*PACA, pancreatic cancer; VDR, vitamin D receptor

N-acetyltransferase 1 (NAT1) rather than NAT2 seems to be predominately expressed.

Germ-line disorders

A number of germ-line disorders are causally linked to pancreatic cancer. These include: familial pancreatic cancer syndrome, mutations in the BRCA2 gene, familial atypical mole and melanoma (FAMM) syndrome, heredity non-polyposis colon cancer (HNPCC), familial adenomatous polyposis, Peutz-Jeghers syndrome, ataxia-telangiectasia, cystic fibrosis, Li-Fraumeni syndrome, and hereditary pancreatitis. To date, there is insufficient evidence to determine whether there is a diet–gene interaction for any of these diseases. But it is likely that patients suffering from these germ-line disorders also carry the same polymorphic detoxifying genes present in the general population and, if so, there could be complex types of gene–gene–diet interaction.

Chronic pancreatitis

Chronic pancreatitis (CP), a disease often caused by heavy alcohol intake combined with smoking, has been identified as a precursor of pancreatic cancer. Patients with CP have about a 15-fold increased risk of pancreatic cancer (Lowenfels *et al.,* 1993). It is therefore possible that different genotypes of alcohol-metabolising enzymes, such as alcohol dehydrogenase (ADH) or aldehyde dehydrogenase (ALDH) might be related to development of pancreatitis and, indirectly, to the subsequent development of pancreatic cancer (Maruyama *et al.,* 1999).

Hereditary pancreatitis

Hereditary pancreatitis is a rare disease which resembles alcoholic pancreatitis except that the disease has an early onset, usually before age 20, and multiple generations are afflicted. Mutations in the trypsinogen gene on chromosome 7q35 have been discovered and are causally related to hereditary pancreatitis. Although the trypsinogen gene is not a cancer gene, patients with hereditary pancreatitis have a risk of pancreatic cancer about 50–60 times greater than normal persons (Lowenfels *et al.,*1997).

We have recently detected a strong additional effect of smoking: the risk of pancreatic cancer in current smokers with hereditary pancreatitis is approximately 150 times greater than in the normal population. In addition smoking appears to lower the age of onset of pancreatic cancer by about 20 years. Although we have no information about diet–gene interaction in this high-risk group, it seems reasonable to suspect that modifying genes could also interact with the defective trypsinogen gene to alter the risk of cancer.

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2.3 Genomics

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Sequencing projects

Rapid developments in sequencing technology have led to an explosion in the amount of DNA sequence data available to the researcher. Mass sequencing projects have been concentrated in two areas: the whole genome, of which the largest ongoing project is that of the entire human genome, and expressed sequence tag (EST) libraries.

Value of EST collections to the researcher

EST libraries are collections of cDNA clones made from RNA of various sources by priming from the polyA$^+$ tail of mRNA. Thus EST clones contain protein coding sequence of varying length for individual genes. Sequencing projects that systematically sequence different libraries (collections) of ESTs identify many thousands of clones corresponding to different genes. These clones can be used directly as targets for the study of differential gene expression in biological systems. This is not the case for clones from whole genome sequencing projects where the analysed sequence is of long stretches of DNA, most of which does not code for protein products.

Many laboratories involved with the sequencing of EST libraries have made their clones available to the research public through the IMAGE clone collections primarily held at the Lawrence Livermore Laboratory (California). These can be searched over the World Wide Web by a variety of means, and then ordered, allowing researchers to build up their own clone sets.

Microarray technology

The use of such EST clone sets relies on microarray technology: high density spotting of DNA clones onto a solid substrate, on which they can be hybridised against labelled cDNA derived from an experimental source. Globally labelled cDNA from an RNA source is known as a complex probe. The individual ESTs on the solid substrate are targets. The solid substrate used to hold the clones is usually nylon or glass. Glass allows a higher density of clone spotting and this format is known as the microarray. In this format many thousands of target sequences can be simultaneously hybridised to the same complex probe, and thus the expression of many thousands of genes can be simultaneously analysed.

Microarray information and its applicability

An experiment using a microarray yields gene expression information at two levels. At the lowest level information is given about the differential expression of each gene in a microarray in the experiment. This can, when pieced together, reveal the networks of activated and inactivated genes and, for example, the mechanism of action of a toxic chemical. At a higher level the pattern of differential gene expression represents a fingerprint which may be used as an identifier of the tissue involved, its pathology, exposure to a chemical in the diet or from elsewhere or any perturbation giving rise to altered gene expression. Thus a microarray can potentially be used as a tool for analysis of chemical exposure or type. Analysis of differential gene expression may then indicate unexpected common responses to toxic agents.

The MRC Toxicology Unit programme

A microarray group has been set up at the MRC Toxicology Unit, Leicester (http://www.le.ac.uk/cmht/twg1/array-fp.html). A robot has been built to produce the microarrays and clone collections have been constructed from the IMAGE collections. These are being used both in experiments on the differential expression of many individual genes and also for gene expression fingerprinting. The Toxicology Unit team is also engaged in the production and picking of new cDNA libraries in order to produce EST clones from genes that are not currently represented in the IMAGE collections.

The theory and technology of genomics can be demonstrated through a study in human cells with acquired and intrinsic resistance to chemotherapeutic agents using a 4300 clone human known gene array. Analysis of these cells has revealed altered expression of some genes which was expected, for example of the Multidrug Resistance 1 gene, and some instances that were not expected, but which could be associated with phenotypic features of the cells. The microarrays can also be used to analyse gene amplification and deletion through the labelling and hybridisation of genomic DNA.

2.4 Proteomics

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Proteomics is the characterisation of patterns of gene expression at the protein level. Many factors affect gene expression and environmental and nutritional factors may be amongst the most important influences. An important aspect of proteomics is the identification of proteins whose expression levels have been altered. In this way proteomics offers a tremendous opportunity to identify genes relevant to nutrition from the 80–100 000 genes encoded in the human genome. The first step in proteomics is the separation and display of proteins, usually by two-dimensional (2-D) gel electrophoresis. This also allows the relative abundance of the separated proteins to be examined. The second step is the identification of the separated protein spots. Advances in mass spectrometry (MS) have made possible the identification of large numbers of gel-separated proteins at minute levels. Therefore MS is the enabling technology for proteomics and much effort has been devoted to using MS to reveal associations between proteins resolved from highly complex mixtures and sequences in ever-expanding databases (reviewed in Abbot, 1999).

Protein identification by mass spectrometry

The most commonly used strategy for protein identification by MS is peptide mass fingerprinting. The mass spectrometer is used to determine accurately the masses of a few tryptic peptides derived from proteolytic digestion of a target protein. Then these masses are compared with theoretical lists of peptide fragments calculated from databases of known protein sequences. Measurements of peptide masses are most easily performed by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometers. Only a few (four to five) peptide masses were required to identify a protein of known sequence when peptide mass fingerprinting methods were first described (Henzel *et al.*, 1993; Mann *et al.*, 1993; Pappin *et al.*, 1993). As databases have increased in size, the quantity of data required to identify a single protein has increased similarly. Reliable identifications by peptide mass fingerprinting require both an increasing amount of peptide mass data and mass measurements with accuracy of 20–30 parts per million (Jensen *et al.*, 1997). Uncertainties in the identifications made by peptide mass fingerprinting can be reduced if more complicated MS experiments are performed. Tandem mass spectrometers, such as triple, quadruple or quadruple time of flight (Q-TOF) instruments enable amino acid sequences to be determined. In these experiments a mixture of peptides is introduced into a tandem mass spectrometer by nano-electrospray, a highly miniaturised form of electrospray ionisation. Peptides are separated on the basis of their mass by the first mass analyser and then selected peptides are fragmented by collision with argon molecules. A second mass analyser records the resulting peptide fragments. Partial amino acid sequences are obtained by interpretation of these spectra. A target protein can be identified with as little as two or three amino acids of sequence if this information is combined with other data such as tryptic specificity and mass data to generate a peptide sequence tag (Mann & Wilm, 1994). These highly specific sequence tags are required to make identifications of proteins from incomplete or error-prone databases such as those containing information on expressed sequence tag DNA clones and also for the analysis of novel proteins. In most laboratories a layered approach to protein identification is adopted with all samples analysed initially by MALDI-TOF MS. Analyses with tandem mass spectrometers, which require more time and expertise, are conducted only on those samples not already identified or where the identifications are uncertain or ambiguous.

Automation of protein identification

The application of proteomics has placed a great emphasis on protein identifications with high sample throughput. This requirement can only be satisfied by automation of the protein identification process and substantial progress has been made in automating each of the steps involved. Each stage of sample preparation, from 2-D gels to MS, has been automated with the introduction of commercially available 'picker,' 'digestion' and 'spotting' robots. These robots perform the excision of gel spots, the automated proteolytic digestion/extraction of peptides and sample transfer to MALDI targets. MALDI-TOF mass spectrometers are particularly amenable to automation and all stages of analysis by this instrument can be automated. However manual intervention and experience are required for the subsequent evaluation of the comparisons with databases. Automation of tandem MS is more difficult. Newer instruments (Q-TOF) are able to perform tandem MS analyses in a data-dependent manner (i.e. the instrument switches automatically between MS and tandem MS functions based on data acquired during the MS function), although

the introduction of samples by the essential nano-electrospray technique remains a manual procedure. Manual interpretation of tandem MS data has been a major time-consuming factor although automation of this step is in progress. Alternatively databases can be interrogated with the output from raw uninterpreted tandem MS data (Yates *et al.*, 1995) although in both instances the interpretation of matches and data still requires considerable skill.

Two-dimensional gel electrophoresis

The 2-D gel electrophoresis technique is seen as offering a comprehensive view of the proteome. It offers the highest resolution of any protein separation technique and highly complex mixtures containing thousands of proteins can be resolved and displayed on a gel, often without any prior purification. In comparative proteomics the protein contents of cells are displayed on 2-D gels and changes in protein expression levels detected by comparisons between normal and diseased or treated cells. Thus proteins associated with disease, drug treatment, genetic manipulation or changes in metabolism can be identified. This aspect of proteomics has become a key strategy for drug target discovery, identification of diagnostic marker proteins, and the understanding of metabolic and disease processes (Celis *et al.*, 1999).

Comparisons of multiple gels are required to detect significant changes in protein expression levels and a current technical problem is the difficulty in obtaining reproducible 2-D gels. In addition this aspect of proteomics requires large-scale protein identification and a massive investment in automation. However, a more serious weakness in this approach is that only a minority of proteins have properties or abundances that allow them to be detected on 2-D gels. Certain classes of proteins are either absent or underrepresented. These classes include membrane proteins, highly charged proteins, small proteins (less than 10 kilodaltons) and proteins of low abundance. Hence, 'interesting' proteins escape detection (see Urquhart *et al.*, 1998; Futcher *et al.*, 1999). Since genes encoding membrane proteins alone comprise 35% of the yeast genome, it is obvious that a large proportion of the proteome is being ignored by current methods. Inclusion of these absent or underrepresented proteins in the analysis of proteomes requires the use of alternatives to separation on 2-D gels. Some shortcomings associated with analysis of proteins from an entire organism or cell have been described above. Many of these problems may be avoided by concentrating on more simple mixtures of proteins, such as those from organelles and protein complexes.

The mitochondrial membrane proteome

The proteomic efforts of the Dunn Human Nutrition Unit are directed towards the analysis of the mitochondrion. The inner mitochondrial membrane is the site of cellular energy metabolism and contains the protein complexes of the respiratory chain as well as ATP synthase and a family of metabolite transport proteins, exemplified by the ATP/ADP translocase. Other related processes are thermogenesis in brown fat mitochondria, and proton leak, both major energy consumers, as well as cellular Ca^{2+} regulation, maintenance of ion gradients across the mitochondrial membrane, and generation of damaging oxygen radicals. The mitochondrial genome, with its susceptibility to mutation, leading to respiratory chain dysfunction, appears to have a role in the processes of ageing and in programmed cell death (Budd & Nicholls, 1998). In addition, numerous mitochondrial neuropathies and myopathies involving respiratory chain dysfunction have been identified (Wallace 1999, 2000). Many of the proteins responsible for normal mitochondrial functions remain unidentified. Therefore the protein content of the inner mitochondrial membranes is being characterised with the aim of identifying other proteins important to energy metabolism. The categories of 'interesting' proteins include mitochondrial ion channels, which are responsible for the maintenance of matrix ion concentrations. These are low-abundance membrane proteins and, although identified by electrophysiological experiments, have not been characterised chemically. The characterisation of these and similar proteins requires the removal of abundant proteins from mitochondrial membranes and the application of separation techniques suitable for hydrophobic membrane proteins.

The complexity in mitochondrial protein content has been estimated by analysis of the yeast genome. In yeast 989 proteins have recognisable *N*-terminal sequences for mitochondrial import, 442 of these proteins have attributed functions and 108 of the 442 are membrane proteins (Scharfe *et al.*, 2000). The simple analyses of mitochondrial membranes from bovine mitochondria performed so far reveal a greater protein complexity.

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2.5 Basal cell carcinoma: A model system to study how disease phenotype is determined by gene–environment interactions*

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Genetic factors and disease risk

There is much current interest in identifying the moderate and low penetrance genes that determine susceptibility to non-Mendelian diseases including the common cancers (Houlston & Tomlinson, 2000). Because such cancers generally occur in later life and affect life expectancy, recruitment of individuals for family-based studies may be difficult. Consequently, association studies based on a candidate gene/case–control approach have been widely used. However, while a large amount of data has been accumulated for many common cancers, there are few examples of genes that are widely accepted as conferring an altered risk of specific malignancies. Indeed, many studies report contradictory results (Rebbeck, 1997; Strange & Fryer, 1999). These difficulties partly result from poor study design, particularly in terms of the numbers of cases and controls, although there is also evidence that some genes are disease modifying rather than disease causing (Moisio *et al.,* 1998). Thus, some of the discrepant results from case–control studies may reflect differences in the clinical stage of cases from the different centres rather than differences between affected and unaffected individuals.

Cutaneous basal cell carcinoma as a model system

We have considered basal cell carcinoma (BCC) as a useful model in the study of gene–environment influences on disease phenotype. The incidence of these slow growing tumours is increasing world-wide, including in the UK (typically by ~10% per year), indicating that the prevalence of BCC will soon equal that of all other cancers combined. Thus, recruitment of large numbers of cases is readily achieved. A further advantage is that the major environmental risk factor is well recognised to be UV radiation. However, while UV exposure is essential, its relationship with risk is unclear and epidemiological studies suggest only a relatively modest twofold increased risk with increased exposure. The relationship between tumour site and exposure is also unclear; thus, in a significant minority of instances (11.4% in our series), BCC occurs on the less exposed truncal site (Ramachandran *et al.,* 1999).

Between 1993 and 1999 we recruited 1250 patients with BCC from centres in north Staffordshire, Cornwall and Leicester (Heagerty *et al.,* 1994; Clairmont *et al.,* 1999; Ramachandran *et al.,* 1999, 2000). We found substantial inter-patient clinical variability. Thus, patients demonstrated 1–30 primary tumours, a finding that reflects markedly different presentational patterns, rates of tumour accrual and sites of development. There is, at present, no explanation for this heterogeneity although it is unlikely to be just the result of differences in UV exposure. Indeed, accumulating data suggest an influence of host factors on BCC phenotypes. For example, skin type 1, male gender and allelic variants in a variety of genes have been associated individually or, in combination, with increased risk of many BCCs (Heagerty *et al.,*1994; Clairmont *et al.,*1999; Ramachandran *et al.,* 1999, 2000). The genes studied included those that mediate aspects of host response to UV including the detoxification of UV-derived reactive oxygen species, oxidised lipid and DNA products and other potential carcinogens, melanisation, cell signalling and immune surveillance. However, the individual impact (rate ratio 1–3) of these and other allelic variants on BCC phenotype is, like that of UV exposure, relatively modest. Overall, while these data indicate the importance of host factors in determining BCC phenotype, they present a rather confused picture of how many, and in what manner, genes interact to determine tumour numbers and site.

Identification of high-risk subgroups

We proposed that the influence of allelic variants on BCC numbers or site will be most evident in high risk subgroups (Ramachandran *et al.,* 1999). Accordingly, our approach has been to classify patients on the basis of phenotypes such as patterns and sites of tumour presentation and determine which of these categories are high-risk on the basis of tumour numbers. It is then possible to identify genes and host factors associated with such subgroups. It is also possible to determine the importance of UV exposure relative to host factors, in such high-risk patients.

* Not part of working papers for IEH workshop; paper prepared after the meeting in the light of discussions on phenotyping and gene–environment interactions at the meeting.

We have recently focused on patients with the multiple presentation phenotype (MPP), a phenotype characterised by the development of tumour clusters (Ramachandran *et al.,* 1999). A cluster is defined as two or more new, primary BCC. In initial studies in 773 cases, we found that 15.3% had a cluster at the first or a later presentation (MPP). In the remaining 654 single presentation phenotype (SPP) patients, 73.4% of patients had only one BCC (SPP1) and 11.3% patients had more than one presentation but only single tumours on each occasion (SPP2). Clearly, while MPP classifications are fixed, SPP classifications may change with time. Thus, SPP cases may form clusters of BCC or further single lesions during follow-up.

Importantly, MPP cases were high risk: they had more tumours (mean 5.4 BCC) than SPP2 patients (mean 2.4 BCC) even though mean follow-up time (7.6 and 7.7 years) was similar.

Indeed, the range in tumour numbers in MPP cases was 2–30 lesions while none of the SPP2 cases had more than 6 BCC. Importantly, the limited data available on UV exposure indicated that the MPP and SPP cases had received similar exposure (Ramachandran *et al.,* 1999). Our working hypothesis therefore, was that MPP cases inherit a relative inability to manage the effects of even normal exposure to UV. However, few characteristics apart from cytochrome P450 CYP2D6 EM (odds ratio 1.9) and skin type 1 (odds ratio 3), were found to be associated with the MPP and the factors that predispose to this trait remain largely unclear.

We next examined the data for evidence of heterogeneity in the MPP group. We found that while some MPP patients demonstrated only one BCC cluster (single-cluster MPP), others had more clusters (multiple-cluster MPP) during similar periods of follow-up. We found that multiple cluster cases comprised 3.5% of the total BCC population and 22.1% of the MPP group. Not unexpectedly, such cases developed numerous BCC (mean number BCC 11.3) compared with the 113 remaining single cluster MPP cases (mean BCC 3.7) and SPP2 cases (mean 2.4 BCC). They therefore, constitute a high-risk group.

None of the multiple cluster MPP cases reported excessive UV exposure. While the frequencies of characteristics linked with UV sensitivity (male gender, skin type, eye colour) were greater in the multiple than single cluster MPP cases, none of these differences achieved significance. However, the mean age at first presentation with a single BCC was significantly earlier in the multiple cluster group. CYP2D6 was also strongly associated with clustering. The odds ratio for this association, 15.5, was unusually large. This finding together with the association of glutathione *S*-transferase GSTT1 null with multiple clustering (odds ratio 7.4) indicate that while single and multiple cluster MPP cases share a susceptibility to clustering, they differ in the frequencies of inherited characteristics. Thus, cluster cases represent a risk spectrum determined by the relative numbers of risk and protective genotypes.

These data indicate that distinct subgroups of BCC patients are present in the total case group. The multiple cluster MPP subgroup are at risk of developing large numbers of primary tumours and appear to be defined by combinations of risk genes and not by excessive exposure to UV. The MPP may reflect the inheritance of characteristics linked with UV sensitivity and/or an inability to manage the post-exposure effects of UV. Genes encoding proteins with a wide range of functions are therefore candidates. Other subgroups, such as SPP cases who do not have many lesions, may be defined by few risk genes and more UV exposure although this possibility has not been properly studied. Thus, while studies in a total case group may suggest that relevant genes exert a modest effect (odds ratio ~2) on a phenotype, data from subgroups may show much stronger associations (odds ratio ~10) with individual genes or combinations of genes.

Problems and developments in molecular epidemiology

While these studies, based on a total group of almost 1000 patients, show associations (but not causality) between individual genotypes and clinical phenotype, they also demonstrate some of the difficulties in using molecular epidemiological approaches. Thus, because gene/phenotype associations exist in only a minority of the patients, it is difficult to address key questions such as how many genes determine phenotype. We have virtually no information of how and in what numbers, protective and risk genotypes interact to determine a phenotype (Frankel & Schork, 1996). Clearly, multicentre studies based on 5000–10 000 cases are needed to investigate properly the phenomenon of high order gene–gene and gene–environment interactions.

In spite of these considerable difficulties, the use of molecular epidemiological approaches to identify low penetrance genes is acquiring a maturity through the use of better study design and new analytical technologies. Thus, the selection of candidate genes, while often subjective, will be

helped by the use of microarray technology to identify phenotype-specific gene expression. The identification of known as well as unsuspected alleles in such candidates has been facilitated by automated DNA fragment analysis. The use of single nucleotide polymorphisms will allow construction of high density genetic maps and the identification of haplotype distributions and linkage disequilibria to map genes by association methods.

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3 Discussion

3.1 Introduction

3.1.1 Aims

The discussion presented herein has been developed, in consultation with several workshop participants, from the discussions that took place at the workshop on *'Diet–gene Interactions: Characterisation of Risk'*.

A particular aim of the Ministry of Agriculture Fisheries and Food and now the Food Standards Agency in sponsoring workshops such as the one on which this report is based is to stimulate debate and help determine priorities for research programmes on diet and health. Epidemiological studies have provided useful information, but little is known about the cellular mechanisms whereby good dietary practices promote good health and well-being.

The overall aim of the workshop and the discussion herein has been to seek ways, including new research, to move towards a better understanding of the impact on health of diet–gene interactions, in relation to other dietary risk factors, highlighting, in particular, colorectal and pancreatic cancers. The role of diet and possible diet–gene interactions in the aetiology of colon cancer is a particular area of interest. Colon cancer rates are increasing in the UK, which suggests environmental factors may be implicated, but how diet and/or genetics may be involved in colon cancer aetiology is little understood. Pancreatic cancer is much less common than colon cancer but is almost invariably fatal; not only are environmental factors important in the aetiology of the disease, it is also causally linked to germ-line disorders.

In addressing these topics the workshop participants discussed:

- what is known about diet–gene interactions and how current knowledge contributes to characterising dietary risk factors (Sections 3.1 and 3.2);
- new technologies in genomics and proteomics, including mass spectrometry, that could be developed to investigate diet–gene interactions (Section 3.3);
- the integration of epidemiology and toxicology, and the role of biomarkers in characterising diet–gene interactions (Section 3.4); and
- approaches (based on the above) to improving the characterisation of the risks resulting from diet–gene interaction.

Other issues pertinent to the characterisation of risk, including the determination and modification of individual risk, and the provision of dietary advice with its associated public health implications, are summarised in Section 3.5.

Conclusions, based on the papers presented at the workshop (Sections 1.2 and 2) and ensuing discussions, are presented in Section 4, together with some recommendations for future research.

3.1.2 Combined influences of diet and genetics on chronic disease

Doll and Peto suggested that about one-third of all cancer deaths in the USA (range 10–70%) might be related to diet (Doll & Peto, 1981), and 25 years ago Armstrong and Doll (1975) suggested that differences between countries in the incidences of cancers of the colon and rectum were associated with dietary differences, especially meat and fat consumption. Risk factors for colorectal cancer include consumption of meat and animal fat, cooking methods, low fruit and vegetable consumption, alcohol intake, smoking and family

history, but the individual risk factors are much smaller than those for lung cancer.

More recently combined influences of genetics as well as diet on health, and especially on cancer, have been proposed. In particular, individuals with different alleles of polymorphic genes* may have different susceptibilities to both disease and the impact of diet on disease onset and possibly progression. Different polymorphisms may have different influences on different diseases; for example, there is an increased risk of bladder cancer among individuals who are slow acetylators, but an increased risk of colon cancer among fast acetylators.

Potential combined influences of diet and genetics on health, and on cancer in particular, are illustrated by the studies on adenoma and cancer in relation to meat intake (see Sections 1.2 and 2.1), which demonstrate that for individuals classified as fast acetylators colorectal cancer risk increases with increasing red meat intake, but among slow acetylators differences in red meat intake have little effect (Chen *et al.,* 1998). Other studies, such as those on the methylenetetrahydrofolate reductase (MTHFR) polymorphism, alcohol intake and colorectal cancer (see Section 2.1), and on apolipoprotein E (apoE) genotypes, high saturated fat diets and serum cholesterol levels (see Section 1.2), also indicate possible diet–gene interactions and the potentially detrimental effect of combining an 'at risk' genotype with a 'risky' dietary habit.

As outlined in Section 2.2, the possibility of diet–gene interaction may have important implications for pancreatic cancer, which is associated with both genetic and environmental risk factors: about 30% of pancreatic cancer is associated with smoking, 20% with diet and 10% with heredity. Black Americans have the highest risks for pancreatic cancer in the USA (e.g. IARC, 1997), yet no study has indicated any dietary differences between black and white Americans that might account for the difference in risk, and there is little evidence that black Americans smoke more than white Americans (e.g. Kabat *et al.*, 1991). It seems likely, therefore, that the difference in risk is influenced by some genetic component. It is interesting to note that the pancreas and the duodenum are derived from a common embryological tissue and that the duodenum is part of the body with one of the lowest cancer rates (e.g. IARC, 1990), and therefore to consider what protective mechanism, such as the expression on tumour suppressor genes, might have been 'lost' during embryological development.

Following on from studies on diseases associated with an increased risk of cancer, such as hereditary pancreatitis (described in Section 2.2), investigating familial disease clusters may give important insights into possible diet–gene interactions and the relative impacts of 'genetic' and 'shared environment' components of these diseases. Such groups are generally well motivated and could be expected to provide useful information on the efficacy of dietary (or other, e.g. surgical) interventions.

One of the findings from the studies on hereditary pancreatitis is that smoking lowers the age of onset of pancreatic cancer substantially (see Section 2.2); an important question for studies on diet–gene interactions to address might be whether any dietary or lifestyle intervention can have an impact on time of onset and, in particular, help postpone the onset of disease, including cancer. Useful information on the role of diet (or other environmental or lifestyle factors) in disease incidence and progression could also be obtained by investigating similar familial groups (with different diets or genetic make ups) in different countries. In considering studies on familial groups with high disease incidence it is worth reflecting on the relative merits of focusing on small groups at high risk and larger populations at lower risk.

In considering any possible environment-gene (including diet–gene) interactions it is important to know what type of gene is involved. Thus interaction of aflatoxin with p53 to produce a mutated, and therefore non-functional, tumour suppressor gene in a somatic cell (see Section 3.2.1) is an entirely different form of interaction than is a germ-line *N*-acetyltransferase 2 (NAT2) polymorphism interacting with a diet high in food containing heterocyclic amines (see Section 2.1).

The division of cancer susceptibility genes into categories of high and low penetrance is important, and the effects of high penetrance and low penetrance genes should be distinguished. For example, in a situation where a cancer is associated with a mutation in a tumour suppressor gene, a high penetrance gene will carry a very high risk in affected individuals, although the attributable risk in the population may be low owing to the rarity of such genes. A low penetrance gene requires the

* Polymorphism is defined as the occurrence of one or more alternative forms of a gene (alleles) at a frequency of at least 0.01 (any lower frequency is classed as a mutation). The product of variant alleles may have normal activity, reduced or increased activity or no activity. There are ethnic differences in the frequency of particular polymorphisms.

presence of an interacting exposure on which to act. However, while individual risk may be small, the attributable risk for population may be much higher as germ-line polymorphisms are much more frequent in these genes than in high penetrance genes. Thus environmental and dietary factors may be much more critical components of the disease process for low penetrance genes than for high penetrance genes.

3.2 Factors that might affect the characterisation of risk from diet–gene interactions

3.2.1 Lifestyle, infection and age

Several studies of migrants have indicated the potential role of lifestyle factors in the aetiology of some cancers (e.g. Japanese migrants to USA, Haenszel & Kurihara, 1968; European migrants to Australia, McMichael *et al.*, 1980). Dietary differences are important lifestyle differences between populations but, as yet, few of the migrant studies, for example, have included detailed and quantitative dietary investigations. Other factors may be important; migrants may, in some way, differ in susceptibility to the cancers studied. Age might also be a factor; for example, younger Japanese who migrate to the USA experience the same cancer risks as the indigenous western population, whereas older migrants do not. Such uncertainties exemplify the need, described above, to improve understanding of the mechanisms of disease causation.

Studies investigating differences in attributable risks for disease in different populations could provide helpful insight into possible diet–gene (or other environment–gene) interactions. For example, studies in China have clearly demonstrated an increased risk of hepatocellular carcinoma (HCC) associated with both chronic hepatitis B infection and dietary aflatoxin exposure, with a strong interaction between the two (Qian, *et al.,* 1994). Furthermore, in studies carried out among cohorts with similar hepatitis B prevalence, mortality rates from HCC varied with dietary exposure to aflatoxin (Yeh *et al.,* 1989). Thus in these populations a dietary factor (aflatoxin) and an infective factor (hepatitis B) appear to interact to elevate HCC risk. The cell proliferation and/or inflammatory response associated with chronic active hepatitis may increase the likelihood that DNA damage caused by aflatoxin exposure will result in a mutation. A specific mutation in codon 249 of the p53 tumour suppressor gene is associated with HCC, and it appears to be a signature mutation for aflatoxin B1 (e.g. Kirk *et al.,* 2000). It is noteworthy that in another population, that is among Alaska natives, p53 mutations do not appear to be associated with the development of HCC. Native Alaskan men have a higher risk of developing HCC than white US men, and although HCC among Alaska natives is frequently associated with hepatitis B infection, dietary exposure to aflatoxin B1 has not been identified (De Benedetti *et al.,* 1995). Other studies have also found mutation in codon 249 of p53 in DNA isolated from plasma of individuals from a population in the Gambia (West Africa) at high risk for HCC and for exposure to aflatoxin B1 and chronic hepatitis B infection, but not among European patients with various liver pathologies, including HCC (Kirk *et al.,* 2000; see also Section 3.3.2). It would be informative to conduct further studies in Africa on cohorts with chronic hepatitis B infection who have migrated from rural to city areas (e.g. study by Kew *et al.,* 1983), to investigate the influence such a change in environment may have on the liver cancer rate and associated mutation pattern.

The timing of exposure may have important implications for both the influence of diet–gene interactions on health and any dietary intervention to modify risk. Currently there is little epidemiological evidence for the temporal influence of diet on health. Generally most prospective studies investigate people in middle life. In this context the Barker hypothesis (Barker, 1998), which suggests that events either *in utero* or in early life determine disease patterns in later life, warrants consideration.

3.2.2 Individual risk

There is a developing drive to move on from identifying risk for the general population towards determining individual risk and understanding better what modifies individual risk. For example, it is not well understood why 75% of smokers do not get lung cancer, yet such knowledge could not only help in the management of individual risk, but also provide mechanistic information about the aetiology of the disease. The report of the Committee on Medical Aspects of Food and Nutrition Policy (Department of Health, 1998) refers not only to genetics and environment but also the 'play of chance' as contributory factors in the aetiology of sporadic cancers, implying perhaps that each individual cancer has arisen by a unique pathway.

There are many difficulties, both scientific and ethical, in seeking to identify individual risk associated with genetic makeup and then informing individuals of their risk. Nonetheless, where genetic risk factors for disease can be better characterised

overall, individuals can at least be advised as to which risk group they belong to, and if appropriate they can modify their risk by suitable intervention (such as a change in diet or lifestyle; see Section 3.5).

In any event increased characterisation of individual risk associated with genetics will not be practicable until population studies have been undertaken, with sufficient power to identify the relevant genetic risk factors.

3.3 New technologies

3.3.1 Genomics and proteomics

The application of both genomics and proteomics to study patterns of gene expression will facilitate the identification of genes relevant to both nutrition, growth and repair, and energy metabolism and hence provide information pertinent to diet–gene interactions.

As highlighted in Section 2.3, microarray technology is a potentially powerful tool in genomics. It can be used to study the expression of many genes simultaneously, with minimal prejudgement as to which genes are likely to be important to a specific outcome. It can identify candidate disease loci and also patterns of gene expression associated with particular pathologies, toxicities or pharmacologies, or chemical or other exposures (exposure to dietary constituents being just one example). The technique can be used to identify the main gene differences between different phenotypes and would, for example, be expected to be sufficiently robust to identify differences in gene expression between normal cells from different normal humans. The use of microarray methods to investigate changes in gene expression as a function of diet or as a result of exposure of cells to dietary components can be expected to become an important tool in the study of diet–gene interactions.

3.3.2 Use of mutations to investigate the disease process

Another possibility for investigating disease aetiology might be to study mutation profiles in tumours, to see how the profiles vary among different populations and migrant groups, and also to see how they vary according to the stage in life at which critical lesions occur. Mutation profiles are useful not just to study tumours but also to establish background levels of mutations.

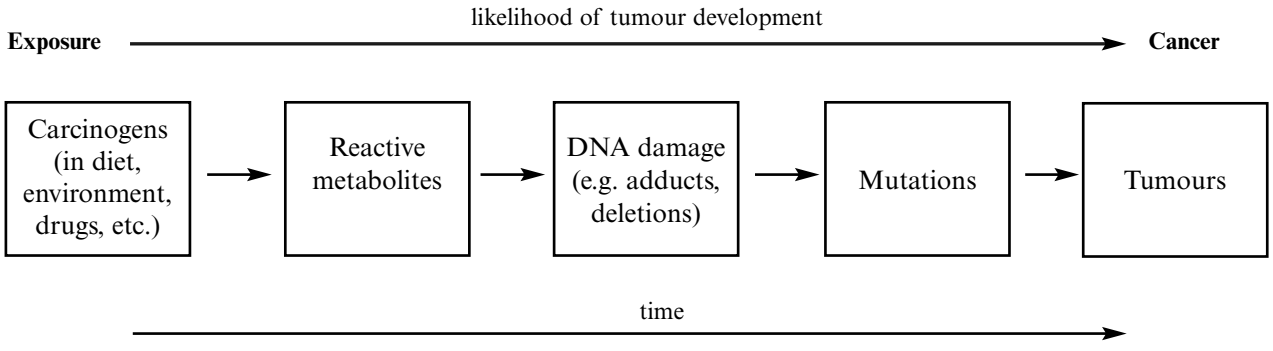
For example, specific mutational changes associated with particular types of chemical exposure have been found in the *ras* gene (Bos, 1989). However, in general, little is known about specific mutations following specific exposures.

The progression from exposure to cancer can be illustrated as shown in Figure 3.1.

Initially, carcinogen exposure was estimated by measuring carcinogen levels in the environment or the diet. As knowledge of mechanisms increased and technological methodology became available, work focused first on carcinogen metabolites in body fluids, such as blood or urine, and finally on the role of carcinogen–DNA adducts. The logical next step will be to study the accumulation, with exposure, of carcinogen-specific somatic mutations; this should integrate both carcinogen exposure and an individual's genetic susceptibility to that particular exposure.

Many of the methods currently used for variant or mutation analysis use hybridisation with specific oligonucleotide probes that can discriminate between wild-type and variant sequences. Such hybridisation can be performed on filters, clip or gels or in solution. These procedures have been successfully used to characterise polymorphisms in metabolic (e.g. P450 enzymes, Worrall *et al.*, 1998) and detoxifying (e.g. glutathione-*S*-transferases, Cheng *et al.*, 1995) enzymes.

Figure 3.1 Progression from exposure to cancer



A group at the International Agency for Research on Cancer (IARC) is currently developing a new approach for looking at carcinogen-specific somatic mutations in body fluids. Using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR), DNA fragments containing an aflatoxin-specific Ser mutation in codon 249 of the p53 gene have been detected in the serum of liver cancer cases and controls from the Gambia, where exposure to aflatoxin B1 is known to be high (Kirk *et al.*, 2000). Occurrence of this mutation has been shown to be more frequent in liver cancer cases or cirrhotics than in controls. It is not known whether such mutational changes are associated only with the presence of tumours or whether they may reflect preneoplastic changes. A research group at IARC is attempting to answer this question by applying a more sensitive and quantitative approach called Short Oligonucleotide Mass Analysis (SOMA), which is based on the mass spectrometic (MS) analysis of short DNA fragments generated by PCR (Lakin *et al.*, 1998). By adapting this approach, through use of an internal standard and digestion of wild-type DNA, low serum levels of mutated DNA can be determined quantitatively in the presence of a high wild-type background. The technique offers the possibility of developing a blood test as an early screen for cancer, which could then be followed up by more detailed investigations, such as those currently practised.

The ability to investigate mutations using new and emerging techniques such as this should facilitate an understanding of the relative influences of genetic susceptibility and exposure in the aetiology of cancers; it should therefore be applicable to the assessment of the impact of diet–gene interactions on carcinogenesis. The goal of such mutational analysis is to investigate tumorigenesis at a much earlier stage than clinical diagnosis, even looking at earlier stages of the process by determining somatic mutations. The earlier the process of carcinogenesis can be detected and assessed, the more it might be possible to benefit the individual.

3.3.3 Techniques for identifying genes of interest

It will be particularly useful to develop feasible, cost effective techniques for identifying the genetic component associated with adverse health outcomes, for use in molecular epidemiology studies.

Many candidate genes (i.e. suspect genes or genes linked to suspect genes) have been proposed and investigated in relation to various biological outcomes but it is not generally clear that they are necessarily the genes that cause the observed effects. Nonetheless, continuing to investigate candidate genes seems appropriate, especially where there is some reasonably biological plausibility for them.

Single nucleotide polymorphism* (SNP) analysis offers a useful way to focus in towards regions in the chromosome around genes of interest. SNPs occur at the rate of approximately 1 in every 300 to 1000 bases. The importance of SNPs is not so much their function (most appear to be non-functional) as the fact that they are markers linked to the genes that are actually important for the biological effect of interest. There is the potential to develop SNP analysis to provide affordable high through-put screening.

One approach to identifying genes associated with an adverse health outcome may be to use pooled samples from diseased and control groups and look for differences in allele frequencies of candidate disease genes, for example using SOMA (see Section 3.3.2); however, such an approach will not give information at the individual level, and more subtle changes may be missed when looking at pooled samples. Conducting such analyses on an individual basis will require considerable resources. In part, future developments should focus on identifying more SNPs and identifying those that are linked to genes with specific biological outcomes that are relevant to the disease under study.

Thus possible technological developments to facilitate identification of specific genes include microarray analysis, using already available SNP chips or custom built chips, and SOMA techniques. SNP chips currently available may not be appropriate as they may not have the required SNPs on them and may be very expensive. However, as the technology develops it will, arguably, only be necessary to use one chip with a full cohort of genes covering the whole human genome, for all studies; this will avoid any bias that might be introduced by developing, for example, a 'diet' chip with all the genes presumed to be of interest to gene–diet interactions, in which case some previously unsuspected genes might be missed. An advantage of the SOMA technique is that it can be used for thousands of individuals in one analysis. It is not yet possible to assess the relative resource requirement and efficacy of these developing methodologies for genotyping, in comparison with traditional methods.

* The most common type of DNA sequence variation, comprising a single base pair difference. It is predicted that there are about 1 million SNPs in human DNA, of which about 50% will be in non-coding regions.

3.4 Role of molecular epidemiology and toxicology in characterising risk from diet–gene interactions

One way to investigate diet–gene interactions is to consider the whole diet and the whole human response, including adaptive physiological or biochemical changes and alterations in gene expression; such an approach may include the use of epidemiological studies. Another, more reductionist, approach is to investigate cell based mechanisms in isolation to identify responses to individual food components or combinations of these. Certainly current epidemiological studies indicate evidence for diet–gene interactions on a population scale, but as yet there is no mechanistic rationale to underpin the findings. A better understanding of the mechanisms of diet–gene interactions is essential for the development of appropriate biomarkers of susceptibility or exposure for use in future epidemiological studies aimed at determining risks associated with diet–gene interactions more precisely. Developments in genomics and proteomics (as discussed above) should help in the better integration of mechanistic and epidemiological studies in the future. One way to link mechanistic studies to responses in exposed humans may be to look at mechanistic responses, in culture, in cells taken from controlled groups of human subjects who have specific identified polymorphisms and have been eating controlled diets. In this way it would be possible to test the hypothesis that a certain genetic make-up modifies the response to a dietary influence.

When considering the genetic component of interactions between diet (or other lifestyle factors or exposures) and genes, it is important to distinguish between genetic factors that predispose to a response to an exposure (e.g. a chemical) and genetic factors that are part of the response, such as changes in gene expression and mutational changes. Both aspects are discussed below.

3.4.1 Genetic polymorphisms

Many different enzyme systems are involved in the metabolism of caffeine, and enzyme differences between individuals arising through genetic and dietary factors will affect responses to it. It is also possible that differences in enzyme activities may influence individual desires for certain dietary components, although this appears not to have been studied.

Fish odour syndrome exemplifies how genetics can influence dietary intake. Certain precursors from fish and some other foods are metabolised to trimethylamine, which is removed by an *N*-oxidation reaction. About 1% of the population are heterozygous carriers of a polymorphism responsible for impaired *N*-oxidation (Rehman, 1999). Individuals deficient in the *N*-oxidation mechanism are deficient in the metabolism of trimethylamine, which has a very potent 'fishy' smell. Once a mechanism is understood in this way, dietary advice to avoid fish and other relevant foods can be provided, as required.

Genetic polymorphisms may affect not only metabolic enzyme systems but also other targets. Polymorphisms of DNA repair enzymes, for example, are particularly relevant to carcinogenesis. The genes for human leukocyte antigens (HLA) are among the most polymorphic class of genes known. Thus variations in immune response are influenced by genetic polymorphisms, and different HLA genotypes are associated with susceptibility to different food allergens; examples are coeliac disease (gluten allergy), peanut allergy and pollen associated food allergies (e.g. Spurkland *et al.*, 1997).

As the majority of the population enjoys reasonable longevity, it seems likely that it is a combination of moderately benign polymorphisms in a number of genes that leads to diseases such as cancer in older age groups, rather than that single polymorphisms have a critical impact in high-risk groups, in which case cancers would be expected to occur much earlier than they generally do.

Difficulties in interpreting the relative roles of genetic polymorphisms and exposure in cancer causation include:

- insufficient data to characterise exposure adequately;
- inadequate definition of disease response;
- lack of genetic characterisation of the polymorphism;
- situations in which environmental factors override the genetic factors;
- the fact that association of a polymorphism with a disease does not imply it causes the disease;
- linkage to other genes whose expression may be more important in the aetiology of the disease;

- the potential for polygenic control and multiple polymorphic influences (e.g. CYP1A1 and GSTM1 in lung cancer).

To date polymorphisms in genes encoding metabolic enzymes have been the most studied. With rapidly evolving techniques it is now increasingly possible to investigate other candidate genes. Identification of the best genes to study is, in part, dependent on biological plausibility; although this has not always been particularly great for some of the gene–response associations currently identified. It may be that, for some outcomes, polymorphisms in genes other than those studied so far actually have more effect; linkage studies may help identify the latter, although it should be recognised that the 'marker' polymorphisms may not actually be linked directly to the disease causing genes. In studying polymorphisms it is important to bear in mind that changes in levels of gene expression can be affected not only by polymorphisms in the gene itself but also by polymorphisms in related genes. It should also be noted that a given polymorphism may not be particularly relevant to a biological outcome because of the modifying effect on gene expression of inducers and inhibitors.

Dietary factors may not have a particularly great impact in a large proportion of the population, but their impact may be important in a minority. The issue is how to identify the minority at particular risk because of genetic factors. Probably alleles of many different genes, individually or together, predispose to a high risk for a particular adverse health outcome and others may modulate that risk. The possibility of identifying genes associated with elevated risks for specific diseases and elevated susceptibility to adverse exposures, including the impact of gene–gene interactions on risk, could be considered a major research area for the 21st century.

3.4.2 Molecular epidemiology to identify predisposing genetic factors

Molecular epidemiology studies such as those described in Section 2.5 provide, in principle, a means to identify high-risk phenotypes (by examining clinical variability) and the genes and other factors (such as environmental factors) associated with them. However, in practice much methodological development will be necessary for such approaches to be widely applicable. DNA screening could be conducted in populations to identify genes associated with particular diseases and hence, ultimately, to identify high-risk individuals who could then be entered into more extensive screening programmes using more traditional clinical investigations.

Rather than concentrating on general population studies, useful information on the identification and role of genetic polymorphisms is more likely to arise by focusing studies on specific subgroups. Groups of patients with a particular disease are generally very heterogeneous, with different combinations of susceptibility genes determining inclusion in particular subgroups. Such subgroups may have a markedly increased risk purely or largely because of their genetic inheritance (e.g. hereditary pancreatitis, Section 2.2) and might require very little of an adverse exposure to trigger illness One example of such a scenario, in a non-food context, might be studying the impact of air pollution on people with asthma.

The studies described in Section 2.5 provide an example of an approach to identify, in a susceptible subgroup (in this case people with basal cell carcinoma, BCC), genes that predispose to a particular phenotype. In these studies, among approximately 1000 people with BCC, the phenotypic variation is very great, and only 32 individuals who carry a very high genetic risk have been identified. However, among these genetically high-risk individuals the odds ratios (OR) for gene–phenotype associations are very much higher (7–15) than those found in typical molecular epidemiology studies (Ramachandran *et al.*, 2001) This is despite the fact that these high-risk individuals appear to have had no more UV exposure than others in the study. Furthermore, tumours appeared in the genetically high-risk group about 10 years earlier than in others in the study.

3.4.3 Genotypes predisposing to risk

Increased understanding of the link between genetic polymorphisms and risk will help uncover other underlying risk factors, such as dietary factors. For example, dividing a study population into subpopulations according to genotype might facilitate the identification of other risk factors by their effects on susceptible subgroups. It is also possible that genotype might influence intermediate disease processes.

However, identifying high-risk individuals according to genotype may not always be feasible, as the impact of genotype may be dependent both on the kind of exposure and the particular disease outcome. For example, in a recent study on cardiovascular disease (Wilson *et al.*, 2000) a genotype that predisposed to tobacco-related cancer seemed to be protective against myocardial

infarction; that is, the genotype elevated one kind of risk but protected against another. Therefore, describing an individual as having an 'at risk' genotype only makes sense in the context of a defined exposure–disease association. In public health terms, therefore, rather than the provision of advice to the individual, the practical applications of studying genetic polymorphism are more likely to be the identification of underlying environmental risk factors, including dietary risk factors, and improved understanding about why genetically susceptible groups are at higher risk.

It is important to note that for sporadic cancers, such as BCC, the development of the neoplasm is not certain, though more likely, in the high risk subgroups. For hereditary colorectal cancer, which comprises 5–10% of colorectal cancer cases, homozygotes for the defective allele associated with impaired mis-match DNA repair have virtually 100% chance of getting the disease at an early age. Heterozygotes need to acquire the second mutated allele, probably through some sort of environmental exposure, that is they acquire a polymorphic allele by a somatic mutation. This is an interesting and potentially informative example of a gene–environment interaction in which an exposure leads to a somatic genetic defect, which in turn leads to the same disease outcome as would have arisen if the defective gene had been present in the germ-line.

3.4.4 Dietary studies

While it is important to develop methodology to identify genes of interest to diet–gene interactions, this will be of little use unless more effective methodologies for evaluating dietary exposure are also developed.

It is difficult to ascribe causality to dietary factors associated with low relative risks (RR), such as those for colorectal cancer associated with red meat consumption (RR 1.5–2; Sections 1.2, 2.1, 3.1.2), particularly when the results are statistically non-significant (confidence interval (CI) including 1), as has been the case in most of the colon cancer/red meat studies. Furthermore, when investigating multiple associations, such as in diet studies, large populations, with a minimum sample size of 4000–5000, are needed to show such low RRs. Nonetheless, although when considering the population as a whole, it has not been possible to find significant associations between diet and gene risk factors and disease, when subgroups have been considered, for example with different frequencies of consumption of red meat or different methods of preparing food, then significant odds ratios (OR) have become evident (see Section 2.1).

In part, associations found between diet and adverse health outcomes may be weak owing to the fact that dietary variables may not be well measured in large epidemiological studies. Based on studies in northern Europe, the correlation between results from food frequency questionnaires and biomarkers (e.g. for protein intake) is generally low unless very detailed measures of intake are used, such as 16 day weighed diet, for which the correlation approaches 0.9 (Bingham *et al.,* 1995). Ideally, studies need to be prospective, as retrospective determination of dietary exposure cannot be sufficiently rigorous; furthermore, improved techniques for recording diet (see Sections 1.2, 3.1) would be facilitated by the development of better food composition tables than are currently available.

Possibly even more important than large scale studies, especially for case–control studies, is to have multiple studies conducted in several centres; one way to reduce the effect of dietary measurement error is to increase the heterogeneity of the study population and hence dietary differences. This is one of the strengths of the European Prospective Investigation of Cancer (EPIC) study, which is the biggest world-wide prospective dietary study to date and can investigate large dietary differences across the European populations studied, especially differences between northern and southern Europe.

However, it should be borne in mind that studies to characterise risks modified by diet–gene interactions may not necessarily be generally applicable. For example, studies by Roberts-Thompson and colleagues (1996) on red meat consumption, conducted in Australia, where meat consumption patterns are different to those in the UK and barbecued meat is more frequently consumed, may not be relevant to an assessment of risk in the UK population. Nonetheless it can be expected that increasing understanding of the influences of extreme differences in factors such as exposure, genetics or diet will facilitate the identification of relevant interactions.

Another great strength of the EPIC study is that, as well as collecting dietary information from questionnaires and data on disease outcome, blood samples are being collected and stored, with a view to investigating physiological variables and examining DNA, in part to study diet–gene interactions.

Although the traditional way to use biomarkers in epidemiological studies has been to conduct a small pilot study first, it may be that for dietary studies and investigations of diet–gene interactions, where it is expected that there will be multiple associations and only a small number of cases or a low occurrence of a biomarker, resources might appropriately be invested from the outset in large scale studies, as described above. This is necessary to avoid the findings of spurious associations. Large scale studies are also necessary for subgroup analysis, which is described above are more likely to identify diet–gene interactions than are general population studies. Nonetheless it should be recognised that, if the developing technologies for genotyping are to be included, at least in their current state of development, such large scale studies will have substantial resource requirements.

3.4.5 Biomarkers

Improved biomarkers of risk are needed to be able to identify those who may develop cancer (or other diseases). Integrating information on genetic polymorphisms and DNA or protein adducts will help to determine the impact of both susceptibility and harmful exposure. Steps involved in identifying those at risk include the identification of:

- genetic factors predisposing to disease, or particular diet–gene interactions;
- harmful exposures (dietary components); and
- responses to dietary components.

The possibility now exists for biomarkers of exposure, effect and risk to be investigated in studies such as the EPIC study. Such investigations will facilitate the assessment of how intrinsic genetic variability is actually affected by dietary factors. Samples taken during the course of the study can be analysed, using conventional and developing techniques, when cases of a specific outcome are identified, and the case samples can be compared with those from different controls and stratified according to the genetic or dietary or other parameters of interest. In this way it may be possible to assess the effects of dietary factors on the genetic material in different target tissues and organs collected. At this level, unless there has been exposure to a genotoxin, the observed effects will not be changes in the genome but differences in gene expression.

Mutations associated with specific dietary factors in human tumour samples may provide one type of biomarker; such lesions have been identified in some studies (as described in Section 3.3.2). The utility of such studies could be enhanced if more mutations in tumours, which are related to particular carcinogens, could be identified.

As well as the more traditional DNA and protein adducts and mutational events that may be used as biomarkers of diet–gene interactions (which when used in the aflatoxin exposure studies identified increased risks that could not be identified by simply using dietary assessment (Qian *et al.,* 1994)), other endpoints, such as receptor occupancy and altered methylation patterns, might usefully be developed as biomarkers of dietary effects.

3.5 Modifying risks

Knowing whether a genetic component is an important risk factor for a disease and understanding risk factors associated with diet–gene interactions may facilitate both the provision of advice to the individual concerning the risk of disease and improved understanding of the disease process. The latter is particularly important as it is often not possible to study disease processes in affected humans. As described in Section 2.2, knowledge about a diet–gene interaction is already used to modify the disease progression associated with phenylketonuria. This exemplifies the fact that from a clinical point of view the goal is to know how best to apply available information to the individual patient. Cancer therapy, in particular, needs also to move towards more effective and lower cost treatments (or interventions). Thus, in principle, increased understanding about diet–gene interactions could lead to suggestions for dietary interventions and help in the targeting of dietary advice. Groups with an identified 'risky' genotype could be advised to modify their diet appropriately.

For example, in response to a high saturated fat diet, serum cholesterol increases more among people with the apoE4 allele (which occurs much less frequently in the population than the other alleles) than among those with the E2 or E3 alleles, with an associated increased risk for coronary heart disease. On the basis of such knowledge, individuals could be assessed for genotype and advised whether saturated fat intake would be a specific risk factor for them. However, in general, evidence on diet–gene interactions is not yet sufficiently conclusive to support such public health intervention. Instead, more research is needed to strengthen the evidence for hypothesised interactions. In the absence of routine gene testing, public health recommendations are still best made at the population level.

Most cancers are not caused by a single dramatic genetic component but by an accumulation of imbalances that eventually tip the scale towards disease. From a public health view point, altering the balance of risk factors enough to halt the progression of a disease, such that it occurs very late in life, if at all, may be a satisfactory outcome. It is not yet possible to modify genes, but the potential to postpone the onset of disease by modifying dietary exposures could have a dramatically beneficial impact on the progression of chronic diseases. It may also be possible to delay disease progression by appropriate dietary modification in people who already have tumours (see IEH, 2001). Some dietary components may have a protective effect, for example by causing apoptosis in the gut and thus killing off damaged cells before they can progress to cancer (Carderni *et al.*, 1998).

Risk factors for disease may vary with age; appropriate dietary advice might therefore vary with age. At early ages it might be beneficial to avoid exposures to genotoxins in the diet, whereas at later ages, avoidance of promoting elements might be advisable.

It seems, intuitively, that it should be possible to ameliorate, by dietary modification, the symptoms of some diseases with a strong inflammatory component, such as asthma, multiple sclerosis and rheumatoid arthritis. It would seem, for example, that the provision of radical scavengers in the diet should have a beneficial effect; however, the influence of dietary antioxidant levels on these diseases has not yet been studied.

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4 Conclusions and recommendations

4.1 Overall conclusions

There is clearly a need to improve understanding about diet–gene interactions as, although current knowledge points to some intriguing indications, there are as yet no examples of clearly identified and characterised interactions. It is important to understand better the effect of diet on genes as well as the effect of genes on diet.

The further development, in particular to improve cost and through-put, of several new methodologies, such us microarray techniques (especially their development for SNP analysis), proteomics and SOMA, will facilitate studies on gene function and also on gene linkage. This will help identify not only genetic factors that predispose to disease or to a particular response to dietary components but also changes in gene expression that are part of the response to a dietary exposure.

Several biomarkers of exposure are already available for use in molecular epidemiology studies. There is a need to develop intermediate biomarkers of risk, for example mutations in key genes, on the causal pathway from exposure to outcome.

Study design is, as ever, of paramount importance. While it is important to develop the techniques to identify genotypes associated with adverse diet–gene interactions, this will be of little use unless epidemiological studies are well designed (as described above) and, in particular, more effective methodologies for dietary analysis are developed. Further research effort should therefore be invested in studies that are well supported clinically, with appropriate identification of exposure and clinical characteristics and properly conducted statistical analyses.

4.2 Possible future research

Based on the workshop discussions presented in Section 3, a number of possible future research directions are proposed.

Studies in migrant populations and ethnic groups within UK, to look at patterns of health, cancer incidence, dietary exposure and genetic susceptibility, should be undertaken, maximising the opportunity presented by the presence of sizeable migrant and ethnic groups in certain regions in the UK. Studies in such UK populations may be particularly informative as some groups now comprise two or three generations, and variations between generations (especially in migrant populations) could be investigated. Studies in migrant populations could also investigate how much age at migration influences disease outcome.

Epidemiological studies on diet and health could investigate populations of different ages, rather than just concentrating, as has been the case until now, on prospective studies in middle age.

Future epidemiological studies should record the most detailed measures of dietary intake as are possible and practical. The development of better food composition tables is encouraged in order to facilitate the accurate production of such dietary records.

Subject to appropriate ethical control, family groups of hereditary pancreatitis patients would provide well-motivated subjects to investigate how diet may affect disease outcome in high-risk groups.

Mutation profiles in specific genes in humans could be used to study the aetiology of disease across different population (including migrant) subgroups. Mutation profiles could also be used to investigate temporal differences in response (i.e. how profiles

vary according to the stage of life at which critical lesions occur), to see if specific mutations can be identified following specific exposures, and to investigate differences in 'background' profiles. The possibility of screening serum for DNA mutations (by e.g. SOMA or quantitative enriched PCR) to identify 'at risk' individuals could be investigated.

Experimental studies could investigate both the relative contributions of diet and polymorphisms in the level of gene expression and activity of, for example, xenobiotic metabolising enzymes, and the extent to which food components can up- or down-regulate protective mechanisms.

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IEH Publications

IEH Reports

Air Pollution and Health: Understanding the Uncertainties	Report R1 (1994)
Air Pollution and Respiratory Disease: UK Research Priorities	Report R2 (1994)
Natural and Man-made Mineral Fibres: UK Research Priorities	Report R3 (1995)
Perinatal Developmental Neurotoxicity	Report R4 (1996)
The Use of Biomarkers in Environmental Exposure Assessment	Report R5 (1996)
Health Effects of Waste Combustion Products	Report R7 (1997)
Factors Affecting the Absorption of Toxic Metals from the Diet	Report R8 (1998)
Recent UK Blood Lead Surveys	Report R9 (1998)
The Non-auditory Effects of Noise	Report R10 (1997)
Approaches to Predicting Toxicity from Occupational Exposure to Dusts	Report R11 (1999)
Benzene in the Environment: An Evaluation of Exposure of the UK General Population and Possible Adverse Health Effects	Report R12 (1999)

IEH Assessments

Environmental Oestrogens: Consequences to Human Health and Wildlife	Assessment A1 (1995)
Indoor Air Quality in the Home: Nitrogen Dioxide, Formaldehyde, Volatile Organic Compounds, House Dust Mites, Fungi and Bacteria	Assessment A2 (1996)
Oilseed Rape: Allergenicity and Irritancy	Assessment A3 (1997)
The Ecological Significance of Endocrine Disruption: Effects on Reproductive Function and Consequences for Natural Populations	Assessment A4 (1999)
Indoor Air Quality in the Home (2): Carbon Monoxide	Assessment A5 (1998)

Special Reports

Understanding Asthma (1995)

Health Effects of Ozone and Nitrogen Dioxide in an Integrated Assessment of Air Pollution (1997)

Fibrous Materials in the Environment (1997)

Organophosphorus Esters: An Evaluation of Chronic Neurotoxic Effects (1998)

Joint Research Programmes on Outdoor and Indoor Air Pollution (Review of Progress, 1999) (2000)

Airborne Particles: Exposure in the Home and Health Effects (2000)

Interdepartmental Group on Health Risks from Chemicals/ Risk Assessment and Toxicology Steering Committee reports

Developing New Approaches to Assessing Risk to Human Health from Chemicals	cr1 (1999)
Risk Assessment Approaches Used by UK Government for Evaluating Human Health Effects from Chemicals	cr2 (1999)
Risk Assessment Strategies in Relation to Population Subgroups	cr3 (1999)
Physiologically-based Pharmacokinetic Modelling: A Potential Tool for use in Risk Assessment	cr4 (1999)
Exposure Assessment in the Evaluation of Risk to Human Health	cr5 (1999)
From Risk Assessment to Risk Management: Dealing with Uncertainty	cr6 (1999)
IGHRC First Report and Forward Plan to 2002	cr7 (2000)
IGHRC Annexes to First Report and Forward Plan to 2002	cr7A (2000)

Food Risk Assessment (FORA)

Probabilistic Approaches to Food Risk Assessment	FORA 1 (2000)
Energy Metabolism and Carcinogenesis	FORA 2 (2000)
Diet–Gene Interactions: Characterisation of Risk	FORA 3 (2001)

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