NUTRIGENOMICS

Report of a workshop hosted by The Nuffield Trust and organised by the Public Health Genetics Unit on 5 February 2004
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The Public Health Genetics Unit (PGHU) was established in 1997 by the Anglia and Oxford, now the Eastern, regional office of the NHS Management Executive. It works in close collaboration with the clinicians and scientists in the University of Cambridge. It is endorsed by the University’s Faculty of Clinical Medicine, and is located at the Strangeways Research Laboratory as part of the University’s Institute of Public Health. It is funded by the NHS through the R&D division of the regional office of the Eastern Region, and by the Bedfordshire, Cambridgeshire, Suffolk and Norfolk Health Authorities.

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FOREWORD

This report on Nutrigenomics marks the latest venture in our theme of exploring the future of health and health services. In 2000 the Trust published Policy Futures for UK Health, a report that explored trends and issues towards 2015 for population health and healthcare in the UK. It identified genetics as being an emerging area where new knowledge and technologies would have a profound impact. In 2000 the Nuffield Trust with the Public Health Genetics Unit in Cambridge complemented this with the publication of a strategic document Genetics and Health that set out the implications of genetics for health and health services and made recommendations on important policy areas.

Much of this work concentrated on the rapidly developing medical speciality of genetics and the implication of new genetic technologies in vastly increasing our capabilities to identify and text for single gene disorders. It also signalled the importance of genes in multi-factorial disease. These are multiple, each individually having a relatively weak effect, and interacting both with each other and with the environment.

The workshop on nutrigenomics, led by the PHGU and hosted by the Trust, began our exploration of this gene environment interaction in the important area of diet and susceptibility to chronic conditions such as heart disease, cancer, obesity and diabetes. Eminent national researchers were brought together with representatives of key policy making bodies to examine the evidence for nutrient gene interaction, to consider clinical applications and to make recommendations for policy implications.

This report sets out the findings from the workshop. The group concluded that the science has great potential to increase our understanding of the molecular mechanisms through which diet influences disease. However, researchers believe that there is no evidence at present to support clinical applications involving individualised dietary advice based on gene testing. Further, they agreed that it would be important to exercise caution in modifying nutritional messages aimed at the public as a whole as this would tend to confuse and dilute the message and would probably be detrimental to the population as a whole.

The Trust thanks Dr Ron Zimmern and the team at the Public Health Genetics Unit for their work in developing the workshop and the subsequent report. It is greatly indebted to the distinguished experts who took time to contribute in preparation of presentations, at the workshops and in the final report.

The Nuffield Trust 2005
1. INTRODUCTION

Associations between diet and chronic disease have long been recognised through epidemiological studies. New genomic technologies are now enabling us to find out more about the basis of these associations through studying the functional interactions of food with the genome at the molecular, cellular and systemic levels and, moreover, the ways in which individuals respond differently to different diets according to their individual genetic make-up.

These two areas, often differentiated as nutrigenomics and nutrigenetics respectively, are potentially of huge importance to the health care system and to the food industry, as well as to those seeking to understand the biology underlying normal homeostasis and disease. There are already many initiatives advancing apace, including the European Nutrigenomics Organisation (NuGO), set up formally in January 2004 and the development of genetic tests purporting to allow individualised dietary advice. Where major diseases of public health importance are concerned the opportunity should be taken to seek out disease prevention where feasible.

In 2003, therefore, the Nuffield Trust asked the Public Health Genetics Unit to convene a workshop to assess the current state of the science of nutrigenomics, in particular looking at any future clinical applications for disease prevention, and to examine arising policy issues.

The half-day workshop brought together researchers taking the lead in this area from some of the main nutrition research institutes in the UK, epidemiologists, and representatives of some of the main funding organisations. The coordinator of the European Nutrigenomics Organisation, Dr Ben Van Ommen, also attended. There were four presentations: Professor John Mathers set out the science of nutrigenomics and Professor Sheila Bingham, Professor Christine Williams and Dr Nick Wareham each provided an assessment of the scientific evidence for gene-diet interaction in chronic disease in their own research area and their view of likely public health relevance.

This workshop followed collaborations between the Nuffield Trust and the PHGU over previous years that had looked at genetics and health policy more generally.
2. THE SCIENCE OF NUTRIGENOMICS

Professor John Mathers

Nutrigenomics covers a wide range of technologies concerned with elucidating how the genetic programme operating in cells and tissues is potentially influenced by diet. John Mathers noted three possible definitions for nutrigenomics:

“… the application of high throughput genomics tools in nutrition research” [Müller & Kersten, 2003]¹

[nutrigenomics] “… seeks to examine ‘dietary signatures’ in cells, tissues and organisms and to understand how nutrition influences homeostasis” [Müller & Kersten, 2003]¹

“… the interface between the nutritional environment and cellular/ genetic processes” [Kaput & Rodriguez, 2004]²

All of these draw certain parallels with other “-omics” sciences, particularly pharmacogenomics and pharmacogenetics, but the comparison cannot be pushed too far; nutrigenomics faces complications that the other two areas do not face, notably the length and the complexity of exposures.

Nutrigenomics has emerged as new technologies, such as transcriptomics, proteomics, metabolomics, and epigenomics have added more complex functional analysis to the basic sequence information provided by the Human Genome Project. Transcriptomics, for example, which uses microarrays, is a very valuable way of beginning to understand how nutritional exposure influences gene expression on a genomic scale. It is possible to group genes of interest for particular metabolic processes and capture information from all of these at once to see how the cell is functioning at any given time or under certain conditions. Such techniques are aided by the commercial development of chips orientated around particular metabolic or functional systems.


2 Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. Physiol Genomics. 2004 Jan 15;16(2):166-77
2. THE SCIENCE OF NUTRIGENOMICS

Proteomics uses protein separation usually on 2-D gels followed by quantification and identification often using mass spectrometric techniques to investigate differential protein expression again under different conditions or with different underlying pathology. The presence or absence of certain key proteins can give information about the early stages of disease.

Metabolomics (or metabonomics) examines global patterns of metabolites present in the cell or in body fluids in response to specific dietary exposures. This requires powerful statistical tools (chemometric analysis) to investigate differences in the NMR spectra or analytes detected by HPLC or other separation techniques.

Epigenetics is the study of modifications to the genome which are copied from one cell generation to the next but which do not involve changes to the primary sequence. These changes, mediated through modification of chromatin proteins such as histones and through the methylation of DNA, contribute to the regulation of transcription and provide a way for the genome to “learn from experience”, regulating gene expression in response to dietary and other exposures and leading to altered cellular phenotypes associated, for example, with chronic disease or ageing.

All of these “-omics tools” have been used to study in detail the molecular responses to food substances or the early stages of disease in common diet-related conditions.

Who will be susceptible to disease and who will be responsive to dietary modification? At the simplest level, we know, for example, that there are differences in nutrient requirements which for several nutrients follow a normal distribution, with some individuals having very high requirements while others need much lower levels than average. At the heart of individual variation is the 0.1% of variation between the DNA sequences of any two individuals. Much of this variation is accounted for by single nucleotide polymorphisms (SNPs), which are largely responsible for differences in complex characteristics such as the way in which we respond to our environment. These differences are likely to apply throughout life, from the early uterine environment right through to different ageing responses later in life.

All of these technologies have so far been applied to cells in culture and to certain model organisms. The challenge will come in applying them to people, where problems both of study design and ethics will be encountered. In addition, with human beings, our means of establishing accurate dietary exposures are imperfect and this is compounded by the need for observations over long time periods, by problems of access to the target tissues and of heterogeneity of target tissues (in other words even small samples will contain a large number of different cell types). At the experimental level, standardisation of assays presents further problems and, finally, there are huge bioinformatics challenges in interpreting the enormous quantities of data generated with further issues in the areas of data archiving and sharing.

Thus, in summary, food components have fundamental influences on health that can be explained by investigation of changes in epigenetic marking of the genome in gene expression, in the translation of messages into proteins and then in the metabolites that are manufactured. If we want to understand how nutrition influences homeostasis so that we can develop better strategies for the prevention (or treatment) of diseases then nutrigenomics offers the kind of tools that might be used.
It is hoped that many of the described problems and issues will be explored jointly across Europe through the newly formed European Nutrigenomics Organisation (NuGO), an EU FP6 Network of Excellence. This organisation will bring together the top nutrigenomics researchers across Europe and provide many of the tools and protocols that will advance this area of science.
Inherited mutations in genes that could be important in causing susceptibility to cancer include genes involved in the metabolism of carcinogens, or in governing hormonal status in hormone responsive cancers, or genes involved in nutrient metabolism. Whilst any individual gene variant probably confers a low risk, this risk may be markedly increased if there is interaction between the gene and environment, particularly diet. Studies of diet in both polymorphisms in low risk genes and in subjects with inherited mutations in high risk genes (such as the APC mutations in the CAPP study) could be important in elucidating aetiology. In addition, there are many direct studies of the mechanisms through which diet causes somatic damage at the tumour site.

*Diet and cancer susceptibility genes.* Although at the cellular level, cancer is recognised as a disease of genes, there is good epidemiological evidence that this is substantially modulated by environmental factors such as diet. In the Japanese population, for example, the rates of colon cancer were low in the 1960s, but since then rates have increased rapidly and now exceed UK rates. This is thought to have been associated with adoption by the Japanese population of a westernised diet, and, possibly, increased susceptibility to it. Evidence also arises from twin studies, where, in a variety of cancers, the concordance rate (probability that a twin will have the same cancer) is greater for monozygotic than for dizygotic twins but generally less than 10%. By implication, therefore, the majority of the risk comes from the environment.

There are, however, a number of challenges for epidemiological studies that aim to elucidate the effects of diet on cancer:

- the aetiology of cancers at different sites varies and so many different nutrients or food constituents need to be assessed
- bias in dietary recall is a serious problem for case-control studies and so prospective studies are needed
- age specific rates even of common cancers are low and for rare sites are extremely low (e.g. around 40 per 100,000 for kidney) and so prospective studies will need to be very large to accumulate enough cases
study design problems are compounded by measurement error both in assessment of dietary exposures and in genotyping.

As an example, the sample size required to investigate a multiplicative interaction between an environmental and an inherited factor would increase greatly with the level of error in the assessment of diet and genotype. This can be illustrated by the numbers of cases required to detect a 2-fold multiplicative interaction for different levels of accuracy of assessment of environment and genetic factors. The number of cases rose from 720 with hypothetical perfect measurement to over 2000 where sensitivity in measuring the environment and genetic factors was reduced to 0.8 and 0.95 respectively. However, very rarely are studies of sizes of this order of magnitude reported in the literature.

One example of a study purporting to show gene-diet interaction was given, but illustrates well the problem of small numbers. The study looked at the relationship between the amount of red meat that people consumed (as a surrogate for heterocyclic amine levels in cooked meat), their genotype for the enzyme that is responsible for acetylating the heterocyclic amines, and their risk of colorectal cancer. The population was split into three risk categories - low, medium and high meat consumption. For fast acetylators there was an increase in relative risk according to the amount of red meat consumed. However, this was a study of only 107 cases and 105 controls, and in the highest risk category there were only 7 cases and 3 controls. The association could well have arisen by chance.

When the amount of fat eaten and breast cancer incidence are compared in various populations worldwide, there is a strong relationship. However, individual risk from fat in breast cancer needs to be shown. The problem of measurement error in the dietary information collected from individuals in prospective studies is well-illustrated by consideration of the many studies using simple methods to assess diet that have been undertaken to assess the effect of fat intake on breast cancer incidence. Generally these have not been able to show that women who eat more fat have increased risk of breast cancer. These and other conflicting results in diet and cancer had led some researchers to suggest that “epidemiology was reaching its limits” with respect to diet, and that resources would be better spent investigating genetic associations in case control studies. However, a study in EPIC Norfolk, which used more detailed and painstaking 7-day food diaries and an accurate method to ascertain portion sizes, was able to show a strong association between dietary fat and risk of breast cancer.

The Norfolk study mentioned above is part of the much wider EPIC study (European Prospective Investigation into Cancer) that also exploits the very variable population and dietary and other lifestyles across Europe to minimise the effects of measurement error and increase the chances of finding dietary associations in epidemiological studies. This is the largest prospective study ever undertaken to investigate specifically the link between diet and cancer. It is multi-centred to ensure sufficient size for even the rarest cancers; it covers much of Europe to ensure that inter-individual variability in food habits is captured, and it is powered to investigate the interaction between dietary and genetic factors. So far, since the project began in 1992, there have been almost 4000 incident breast cancers and over 1000 colorectal cancers. Strong protective effects for fibre in bowel cancer have been shown.
Although genotyping has not yet been undertaken, DNA samples have been collected and will shortly begin to be analysed.

It has been said that genes load the gun but environment pulls the trigger. However, we do not know how environment pulls the trigger. Studies on the relationships between dietary factors and disease risk can begin to shed light on the molecular mechanisms of disease. One example of work in this area was a study of the role of red meat consumption in colorectal cancer. The EPIC study and others have shown that high consumption of red meat increases the risk of colorectal cancer. There are several hypotheses underlying these associations. One hypothesis, that red meat increases the endogenous formation of carcinogenic N-nitroso compounds in the gut, is being studied in carefully controlled intervention studies in humans. To show that such compounds induce accumulation of somatic mutations in key genes associated with the adenoma-carcinoma transition, epidemiological studies relating dietary habits to tumour somatic mutations are being conducted. One study showed strong association between the specific types of mutations associated with the presence of N-nitroso compounds in large bowel tumours and the amount of red meat in the diet. Ideally such studies should be prospective to avoid problems of recall bias in assessment of diet.

Sheila Bingham concluded that dietary risks in cancer are largely not established but are emerging now that there are large biomolecular and epidemiological studies in progress. Researchers need to concentrate on accurate exposure assessments in prospective studies. There are likely to be genes that confer susceptibility to disease through their effect on intermediate mechanisms such as those involved in pathways of nutrient metabolism; this effect may therefore be modulated by diet to either increase or decrease risk. Studies to look at these interactions are in their infancy with current work too often being conducted on small studies lacking power and with huge dietary measurement error.

Work on the effect of diet on somatic DNA damage, or in influencing some of the mechanisms involved in inherited cancer mutations will be important in elucidating mechanisms of cancer development.

Finally, the effect of genetic polymorphisms on influencing individual risk of sporadic cancer from diet and other environmental factors is not established. Risks from variant polymorphisms are associated with different risks at different sites (for example bladder versus bowel cancer). The present current public health advice, to avoid smoking, and consume a high fibre, low red and processed meat diet to avoid bowel cancer, should be given to all individuals. The extent of protection may differ between individuals but, at present, on a population basis, we have no grounds for different advice.
The scale of work needed in the prevention of coronary heart disease (CHD) in the UK was emphasised by Christine Williams. She noted that the rank position of the UK in age standardised death rates has apparently improved in recent years, but that this change in position has been brought about largely by cataclysmic increases in rates of cardiovascular disease experienced by a number of Eastern European countries in economic transition together with, in the UK, the application of drug therapies such as blood pressure lowering or cholesterol lowering drugs.

The vast majority of adults will experience significant decline in cardiovascular function with age and so, as the population ages, there is potentially a large pool of individuals who would require these treatments. It has to be questioned how many of these could be dealt with by a therapeutic model. Nutritionists would promote a dietary prevention model, which states that, when introduced at an early enough age, the appropriate diet will maintain cardiovascular health into late old age with the final decline happening quite rapidly. As well as quality of life benefits, there would be clear economic advantages from avoiding the prolonged use of drugs.

Arguments against a dietary prevention model have been many and varied: that the evidence is not there; the epidemiology is confusing; the messages are confusing; and the population is not convinced that diet is an effective preventive strategy. However, some countries, such as Finland, have implemented effective dietary prevention models with success in lowering coronary heart disease rates.

Another argument is that the response to diet is variable. This can be shown by the response of individual low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol and triglyceride to dietary changes. Whilst the mean response is a modest beneficial change, individual responses may vary from remarkable reductions in some to increases in others. Many people think that the basis of this variation is genetic heterogeneity. There are some genes that will determine a beneficial response to a low fat diet and others that militate against it. The argument is that, if these different genotypes could be identified,
it might then be possible to provide much more focussed advice, resulting in a better response from those individuals who stand to benefit most.

Evidence from different ethnic minorities with very different rates of heart disease despite similar diets also suggests that genetic differences are operating in CHD.

Can susceptibility genes be identified that are responsive to diet? If these gene diet interactions can be identified what relevance might they have to public health messages? Do they indicate a more individual approach to dietary change? This may indeed be the case in the future but the present state of knowledge is very preliminary; any change to existing public health messages would be premature and could do harm.

Some examples were given of genes that might be of interest. The most heavily researched area is that concerned with genes that determine levels of circulating lipid risk factors - lipoproteins, LDL, HDL cholesterol, triglycerides and lipoprotein particles. There are many potential susceptibility genes, particularly, for example, those concerned with producing the proteins involved with the metabolism of these particles. Others might involve the transport of these particles, or the structure and function of target sites. For example, familial hypercholesterolaemia (FH) is caused by a mutation in the LDL receptor that results in a catastrophic increase in LDL concentrations in the blood and an increased risk of cardiovascular disease. This condition is rare and so could not account for much of the disease in the population. However, single nucleotide polymorphisms (SNPs) in genes involved directly or indirectly in lipoprotein metabolism could be important in determining diet gene interaction. Research in these areas has been undertaken for the last 10 -15 years.

A simple example was given to illustrate the principles. APOE is involved in the transport of chylomicrons and VLDL and as the ligand for the lipoprotein receptor-related protein (LRP) and the LDL receptor. It is therefore involved in the transport and uptake of these particles in the circulation. Structural and functional changes in this protein could impact on lipoprotein concentrations. There are three common isoforms with prevalence of E4 25%, E3 65% and E2 10%. We know that individuals who are heterozygous or homozygous for E4 have a significantly higher risk of cardiovascular disease and there is also an association with Alzheimer's disease. They also have higher circulating cholesterol concentrations. It is found that the response to a low fat diet varies with genotype so that those with the E4 genotype show a much larger response. Not all studies are in agreement but the general trend seems robust.

Another example comes from the nurses health study, a large-scale prospective study in which the relationship between APOA1 polymorphisms and circulating HDL cholesterol has been investigated. The AA genotype shows a larger increase in HDL cholesterol (the "good" form of circulating cholesterol) in response to dietary polyunsaturated fat than does the GA genotype or the wild type GG, which actually shows a small decrease in HDL. This difference would be associated with a major change in cardiovascular disease risk.

In both of the examples given so far, all genotypes benefit from the dietary intervention but some show a larger response than others. It is more problematic if some individuals show an adverse response. For example, a study of the effect of fish oil supplements for a group of men showed different responses according to E2, 3 or 4 genotypes of the APOE protein. Whilst
E3 and E4 groups had beneficial responses, the E2 group showed a 15% increase in LDL and significant reduction in HDL, two responses that would be deleterious. Thus, for this group, general public health advice was potentially inappropriate. The study itself, however, could not be directly translated to public health action because very high doses of fish oil were used. Further studies, which use lower levels of exposure and, also, recruit people into the trial according to genotype are currently underway.

The implications of diet for lipoprotein-genotype interactions were summarised by Christine Williams as follows:

- Beneficial changes are more marked in some individuals than others
- Some healthy diet recommendations may have deleterious effects for some genotypes
- The implications for generalised dietary advice to populations are unclear

Christine Williams ended by summarising her main conclusions about the current state of science and its implications for clinical and public health practice:

- There are diet genotype interactions; some individuals respond to certain dietary changes more profoundly than others.
- Attempts to look for candidate genes through association studies are likely to be confounded by diet if dietary exposure is not measured accurately.
- If polymorphisms where there are significant diet gene interactions can be identified, then this may also give information about the mechanisms underlying the relationship between diet and disease.
- There are considerable benefits in pursuing this line of research but also risks in trying to apply it to public health too soon and before the scientific evidence is robust.
- There are questions about whether individualised advice will be feasible. There are many ethical considerations, and it may be that consumers will reject the concept of genotype analysis. There will be a need for more applied research in this area, possibly funded by the food industry who might become involved in a way similar to that of the pharmaceutical industry in pharmacogenetics.
5. GENES, DIET AND DIABETES

Dr Nick Wareham

Diabetes and obesity are conditions where researchers believe diet gene interaction is likely. Nick Wareham described these as very good models for study but emphasised that, even in these common conditions, there were still substantial difficulties. Further, he firmly took the position that the study of gene-diet interaction in diabetes should not be allowed to confuse current public health prevention messages.

There is evidence from migration studies, cross sectional studies of geographical variation and temporal variation all pointing to the suggestion that the basis of diabetes is fundamentally an interaction between genes and the environment. This is of prime importance in understanding the mechanisms of disease rather than in altering any current public health advice. There is already good evidence that diabetes can be substantially prevented by changes in diet and increasing physical activity. Current lifestyles predisposing to diabetes are a societal problem and need to be tackled at this level rather than at the level of the individual and it could be counter-productive if some individuals gained the impression that genetic differences might make them less susceptible to diabetes and thus not at risk from whatever lifestyles they chose.

The concept of gene-nutrient interaction poses an immensely interesting scientific question. Taken at the level of epidemiology, it raises the question: how do we get from evidence that there may be an interaction to a greater degree of certainty? The nature of epidemiological evidence is one of slow accumulation and will be difficult to achieve. The sort of evidence that is required is:

- a functional variant leading to a clinical outcome
- an environmental or dietary factor predicting a clinical outcome

For either of these, evidence of relationship to an intermediate quantitative trait would provide additional support.

However, study design problems mean the evidence for dietary factors relating to clinical outcome is weak. For example, the relationship between diet and type II diabetes in terms
of epidemiological evidence is much weaker than people often realise, principally because of problems with study design. The ideal design is a nested case control study - that is, a case control study nested within a prospective study- because it avoids the problems of recall bias. Studies need to be large because of difficulties in achieving precision. At present, there are only a handful of these types of studies in the literature. For example, the Cambridge group has recently published a study on the relationship between polyunsaturated and saturated fats in the diet and incident diabetes.

The addition of genetic factors adds a further level of complexity. Some progress has been made in identifying single-gene causes of obesity. For example, mutations in the MC4 receptor gene are associated with severe obesity in some families. However these mutations are very rare and no relevance to the more common polygenic forms of obesity has been demonstrated.

Research has been somewhat more fruitful on genetic factors underlying diabetes, with evidence arising from linkage studies and association studies. There are some monogenic cases of diabetes, mostly maturity onset diabetes of the young (MODY), and genes associated with this condition have been identified. Whole genome scans have identified several genomic regions that are likely to harbour genes contributing to polygenic forms of diabetes and a number of candidate genes have been studied, including the PPARgamma and calpain genes. Although this area of research is promising, the evidence remains weak at this stage.

Each of the genetic variants implicated in type II diabetes seems neither necessary nor sufficient on its own to cause disease; a “second hit” is needed that could be another genetic mutation or an environmental factor. The PPARgamma gene could be a key candidate for involvement in a gene-environment interaction. PPARgamma is a nuclear receptor that is involved in lipidogenesis and lipid metabolism. Certain rare mutations in the gene encoding PPARgamma cause insulin resistance, and there are more common polymorphisms that affect insulin sensitivity. Evidence is building from an understanding of molecular mechanisms that PPARgamma might be important for gene-diet interaction, for example the natural ligand of the receptor is a fatty acid, and the degree of affinity of the fatty acid for the receptor varies according to the length of the fatty acid and the degree of saturation. However, the epidemiological evidence for an association of this gene with diabetes is mixed. A meta-analysis carried out by Altshuler et al showed that the common PPARgamma Pro12Ala polymorphism is associated with a small degree of protection against the disease (OR=0.8).

In order to detect an effect of a gene that interacts with a dietary factor to cause a disease outcome, extremely large and prolonged studies are needed: probably in excess of half a million people followed up for fifteen years or more. There are three main reasons for the need for such large numbers:

- odds ratios will be relatively small
- measurement errors for diet are worse than generally estimated
- multiple genes will have to be tested and so lower p values have to be obtained to demonstrate statistical significance

In the long run, evidence for gene-diet interaction will slowly accumulate from the synthesis of evidence from many different studies. Quantitative trait studies can make a valuable contribution (for example, there is good evidence that people with different genotypes for PPARgamma have different fasting insulin levels in response to dietary fat), but it is difficult to make the transition from such quantitative information to the discrete clinical outcomes that are of interest to patients and clinicians.

Evidence from observational studies such as Biobank or EPIC will not be enough on its own to detect gene-environment interactions but will need to be supported by aetiological trials involving analysis of differential responses to a changing environmental factor. Such studies can provide a stronger causal inference than observational data. Some studies of this type have been done but they have generally been too short-term and have tended to investigate a general lifestyle intervention, whereas quite specific interventions will be required. Newly designed trials will need to enrol people on the basis of genotype; a key factor in achieving the numbers needed will be the allele frequencies for the genotypes of interest. The selection of participants on the basis of genotype may also raise ethical issues.

In summary, robust evidence supporting a suggested gene-diet interaction will include:

- Association of a functional variant and an environmental factor with outcome
- An a priori biological hypothesis that supports the search for an interaction
- Functional studies in animals
- Evidence from linkage studies
- Aetiological trials
- Study replication
- Studies of adequate power

Nick Wareham concluded by considering whether studies of gene-environment interaction have any relevance now. There are a few examples that provide preliminary encouragement. For instance, there is evidence that the genotype at the GPR10 gene is at least partly responsible for differences in response to attempts to lower blood pressure by increasing physical exercise. Testing the GPR10 genotype might aid the choice between drug intervention and lifestyle advice.

Information from family history could also be used more proactively than at present. For example, the combination of obesity and a family history of diabetes is known to be strongly predictive of diabetes; those with such a family history would have much to gain from controlling their weight.

This example raises important questions regarding behaviour, motivation and risk perception. How does knowledge of a fixed risk related to family history or genes affect people's response to being advised to change their behaviour to reduce their risk of disease? We do not know whether, and for whom, such information would be a motivator or would create a counter-productive feeling of fatalism.
6. DISCUSSION

The need for caution

The science of nutrigenetics and nutrigenomics is exciting but the extent to which it can be translated into applications in mainstream health care, and how far in the future such applications may be, are unknown at present. The overriding concern of the workshop was the need to exercise caution and not to promise too much too soon.

The promotion of healthy patterns of nutrition at a population level is of paramount concern for public health. Key messages on a healthy diet are well established and we should not risk diluting these messages with premature speculation about the impact of nutrigenomics. There is also a danger of raising unrealistic expectations, which could lead to a public backlash and could jeopardise research funding if the science is perceived as failing to deliver.

“We must not get into the situation of confusing the public as they already do not trust nutrition information.”

The consensus of the meeting was that it was much too soon to develop any new ‘healthy eating’ messages derived from nutrigenomics research. At the individual level there is a danger of scaring people about increased risk. The polymorphisms that we investigate will be likely to be common and convey an increased risk of disease at a population level, but for the most part they will not be of sufficient importance at an individual level to warrant specific dietary advice (although there will be subsets of people at very high risk for whom dietary advice may be appropriate).

The group noted that genotyping as a basis for delivery of personalised dietary advice is currently available over the Internet. Whilst there was considerable scepticism about the robustness of the evidence underpinning this activity, it was not thought that the level of activity in the UK was sufficient at present to justify the issuing of public advice.
Nutrigenomics and nutrigenetics

The distinction between nutrigenomics and nutrigenetics provided a useful basis for discussion about what the new technologies could do and the problems facing future researchers and those concerned with the development of health applications. Nutrigenomics uses the new "omics" technologies at the level of molecular and cellular processes and biological systems and can tell us how things work. The new technologies have allowed us to take account of the whole process, to identify cell signatures which represent exposure to the environment and to investigate the earliest changes in disease processes.

Nutrigenomics could make contributions to the study of human nutrition at many levels. For example, it could help to define safe upper and lower limits for essential nutrients and micronutrients. By identifying key genes involved in dietary responses, nutrigenomics can also give some indications of those genes in which polymorphisms might be important and these may then be explored further in epidemiological studies. The study of these individual variations, their interaction with nutrition, and their association with health and disease, is the complementary study of nutrigenetics. This end of the science may be more readily translated eventually into public health applications, but is fraught with many unknowns and levels of complexity.

Until recently, nutritional science has suffered from the inability to understand mechanisms of diet disease relationships. Genomics and the post-genomic technologies offer the opportunity to understand the roles of food components at the cellular and molecular levels, and in particular their interaction with the genome, that will strengthen nutritional science.

The developing science: difficulties and opportunities

The measurement of dietary exposure

The accurate measurement of dietary exposure will be fundamental if we are going to understand the interaction of nutrition with the genome. The limitations of currently available tools such as dietary questionnaires were emphasised. This is a problem that must be tackled. The large prospective cohort studies such as Biobank and EPIC provide an opportunity both to use the best tools available for assessing exposure and to develop and validate new approaches that might be more robust. Molecular approaches, such as measurement of epigenetic changes that act as markers for exposure over long time periods, might be helpful in validating exposure measurements based on self-reported dietary history.

Quality assurance and standards

Quality assurance in the research laboratories involved in this new research area is not yet sufficiently well-established. There is a need to develop guidelines and standards, which incorporate the state-of-the-art and enable and encourage researchers to apply the 'omics' technologies in ways that can be replicated, so that comparisons between laboratories can be made and which facilitate pooling of samples and data. These are all issues which are at the core of the integration activities of NuGO.
Genotyping also requires rigorous quality control, especially when dealing with rare alleles. Even with more common alleles such as the APOE variants it may become an issue when fast throughput techniques are employed, or when it is necessary to bring study samples together for meta-analysis or further biological analysis.

The Group accepted that research laboratories could not be regulated as tightly as clinical laboratories - they could not, for example, be required to use standard protocols. However, when samples were submitted, the researcher should be entitled to know that internal processes were in place to ensure high quality data. It was also useful that funders such as the BBSRC (Biotechnology and Biological Sciences Research Council), DEFRA (Department for Environment, Food and Rural Affairs) and the Food Standards Agency (FSA) are in the process of developing guidelines that will require quality assurance of laboratories. These funders are also concerned about sample storage and archiving. Quality assurance has also been an important issue in the Biobank discussions recently. It is also a major theme of the new European Nutrigenomics Network (NUGO), where, for example, there is work to standardise protocols for the machinery available in the 21 member universities and research institutes so that comparable assays can be run throughout the network. There are also useful parallels with the MIAME (Minimum Information About a Microarray Experiment) initiative4 in the area of microarray studies of gene expression: key researchers in this field have developed a protocol for recording how studies are done so that they can be compared between laboratories and over time, and potentially reproduced. NuGO has taken up the issue of creating a “MIAME nut” version of data acquisition and reporting for nutrigenomics data.

**Epidemiological studies**

Nutrigenetics, which builds on data arising out of new “omics” technologies, is moving us into a new era of nutritional epidemiology. It brings with it immense complexity and unprecedented amounts of data to be analysed. New techniques will have to be developed, and extreme caution exerted in any attempts to translate findings into public health messages.

It will be important to have large studies, such as the EPIC study, which have good phenotypic markers, and, if possible, repeat measurements of phenotype and biomarkers of diet. There will also need to be epidemiological studies that can investigate gene-gene interaction. It was suggested that there may need for a specific large study, similar to Biobank, but focussed on diet gene interactions, as there may not be enough phenotype markers in Biobank for it to be used for this purpose.

Systematic reviews and meta-analysis will be important in this area although it can be difficult to get funding for such secondary research. Nutrigenetics presents new challenges to epidemiology. New techniques must be developed because of the need to deal with multiple genes and multiple pathways. Ways will need to be found to overcome problems of publication bias and to synthesise the vast amounts of data into meaningful information or

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4 See website www.mged.org/workgroup/MIAME/miame.html
knowledge. Automated meta-analysis has been mooted as a response to this, but the idea causes some disquiet unless it is able to include the mainstays of systematic analysis: namely the importance of scrutinising the quality of studies. Thus developing new tools and a framework for meta-analysis and systematic reviews will be critical.

The lack of replication of study findings is often related to limited study power. Opportunities can arise to add genotyping to other large epidemiological studies. However, when limited resources are available such additions might not be funded.

Genomics techniques can help us to study further the variability in gene-disease relationships reported in the literature. Attempts to understand these relationships are often obscured by the fact that dietary exposure effects are not accounted for. Once these are brought into the equation it can become clear that the way in which a particular polymorphism is associated with disease depends on the dietary exposure.

Similarly, the application of genomic techniques is likely to prove a powerful approach to mechanistic understanding of diet-disease association and there are potentially huge advantages from pooling functional information from nutrigenomics investigations with epidemiological information.

**The development of public health action**

_“There is a rather naive view of the word translation that implies a very linear process”._

The relationship between science and public health is not straightforward and managing change in this area is not well understood. We need to understand better how change can be managed in order to move forward.

**Capacity**

Problems of insufficient capacity are already emerging for research and, potentially in the near future, will become important for practice. There will be a need for more epidemiologists, biostatisticians, specialists in informatics, and nutritional scientists able to work in nutrigenomics. To some extent this is impossible to plan for as it will be dependent on how the science evolves and the amount of funding made available.

Practitioners will need to be able to address this area within both clinical practice and in their public health work. As nutrition becomes recognised as an important determinant of health, and increasingly dietary change is seen as a first line strategy in dealing with common conditions such as obesity, hypertension and hypercholesterolaemia, there will be increasing recourse to dietitians within health services. The content of a wide range of professional courses, including medical courses, should be expanded to include more teaching of nutrition. There may be a need for a specialty of public health nutrition, whose practitioners could integrate population-based advice with new approaches arising from the evolving science of nutrigenomics and nutrigenetics. It was noted that the Nutrition Society had developed a Register of Public Health Nutritionists whose members had explicit training and expertise in this area.
Policy and ethics

The workshop touched on three important ethical issues that need to be addressed:

*Collecting genetics information for policy and research.* It is likely that the application of nutrigenetics will raise ethical issues some of which are similar to and already being tackled by, pharmacogenetics. The experience of pharmacogenetics will therefore give an indication of whether people are prepared to allow use of their genetic information for the purposes of research and, ultimately, treatment.

*Will we create populations of worried well?* Is there a danger that genetic susceptibility to diet-related disease will come to be seen as equivalent to the disease itself? This problem is not limited to nutrigenetics but is part of a more general question of what constitutes health and disease in the context of predictive genetic information.

*The problem of researching on people with specific alleles.* The issues around research studies involving selection of individuals on the basis of their genotype need open discussion and dialogue with ethics committees. Often the best solution is agreement not to release genotype information to the subjects or to any third party.

Funding

Funding in this area has increased in recent years. For example, BBSRC noted that funding for application of high throughput genomics in the diet and health area increased from £1.5 m to £2.2 m between 2001 and 2002/3 and if studies looking at the effects of diet on interaction with a single gene were included, the increase was from £3.5m to £4.1m so this is one of the most rapidly expanding areas. Increased funding would increase the pool of trained researchers.

NuGO

NuGO is a Network of Excellence funded through the European Union’s 6th Framework programme. The money is primarily for integration purposes; there are 21 research institutes and universities involved and the money supports research collaboration, standardisation, training and communication. The European Bioinformatics Institute is one of the NuGO partners and has taken the lead in development of the nutrigenomics version of MIAME in order to collect and store “omics” data. The Institute for Food Research at Norwich is taking the lead on collecting information on ‘omics’ facilities available in each of the participating laboratories so that each can run to standard protocols.

The programme includes a number of work packages (“Joint Research Activities”), which are focussed on the areas:

- gut health and function
- metabolic health
- life course nutrition
- risk-benefit evaluation, which aims to define both the optimal intakes and safe limits of consumption of bioactive food components.
There are also activities within an area called “Spreading Excellence”, which aims to build on acquired knowledge and share this with target groups such as other European researchers, industry, society and healthcare workers. Finally, there is a small amount of money now available for collaborative research.
The following were agreed as the key recommendations arising from the meeting:

1. A Report should be produced which summarises public health implications in this area. It should urge caution in promising too much from this very new science too soon and should emphasise the need not to confuse population public health messages.

2. This expert group should make a public statement about balancing the need for caution against potential excitement of the science and future possibilities for health applications.

3. Communication of the emerging science with the public should be pursued in order to develop and maintain public support in this area. Journalists would be a key group for this, and the possibility of a nutritional “Centre for Life” was raised.

4. Collection of exposure, phenotypic and genotypic data should be promoted and improved with common protocols to enable comparable data to be collected and stored. This will require a better infrastructure including standardised protocols and databases.

5. Funders and researchers should demand more robust assessment of dietary exposure and nutritional status within epidemiological studies and the collection of suitable biological samples for nutrigenomics studies.

6. Those responsible for education of health professionals should aim to increase the level of genetic and genomics literacy amongst public health professionals and nutritionists and enable them to eventually incorporate nutrigenomics and nutrigenetics concepts into their practice.

7. Thought should be given to the ELSI issues around individualised advice. The focus should first be on situations where mainstream advice could be deleterious for some subgroups.
8. APPENDIX

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