

Workshop Report

Nutrient–gene interactions in benefit–risk analysis

John Hesketh^{1*}, Iwona Wybranska², Yvonne Dommels³, Maria King¹, Ruan Elliott⁴, Catalina Pico⁵
and Jaap Keijer³

¹*Institute of Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, UK*

²*Collegium Medicum, Jagiellonian University, Krakow, Poland*

³*RIKILT – Institute of Food Safety, Wageningen, The Netherlands*

⁴*Institute of Food Research, Norwich, UK*

⁵*University of the Balearic Islands, Palma de Mallorca, Spain*

(Received 21 November 2005 – Accepted 21 November 2005)

Individuals respond differently to nutrients and foods. This is reflected in different levels of benefits and risks at the same intake of a nutrient and, consequently, different ‘windows of benefit’ in terms of nutrient intake. This has led recently to the concept of ‘personalised nutrition’. Genetic factors such as single nucleotide polymorphisms may be one source of this inter-individual variation in benefit–risk response to nutrients. In 2004 a European Union-funded network of excellence in the area of nutrigenomics (European Nutrigenomics Organisation; NuGO) organised a workshop on the role of nutrient–gene interactions in determining benefit–risk of nutrients and diet. The major issues discussed at the workshop are presented in the present paper and highlighted with examples from the presentations. The overall consensus was that although genetics provides a new vision where genetic information could in the future be used to provide knowledge on disease predisposition and nutritional requirements, such a goal is still far off and much more research is required before we can reliably include genetic factors in the risk–benefit assessment of nutrients and diets.

Nutrient–gene interactions: Nutrigenomics: Benefit–risk analysis: Single nucleotide polymorphisms

Eating food represents a major interaction of humans with their environment. Every food that we eat and every nutrient that we absorb from our gastrointestinal tract may have potential benefits or risks. Our nutritional status provides us with a body condition that may help us to combat disease or, conversely, may make us more susceptible to disease. Nutrition is concerned with the analysis of these benefits and risks and nutritionists are continually in search of the diet that will provide the population with optimal health. It is known that a deficiency in certain nutrients increases the risk of disease, and as intake increases this risk is reduced up to the point where intake leads to a health benefit. Ultimately at high intakes many nutrients are no longer beneficial and the risk of disease increases again. This benefit–risk response of nutrient intake is illustrated diagrammatically in Fig. 1 and shows that for a given nutrient there is a range of intake that provides a ‘window of benefit’.

What is equally clear is that the response to a given nutrient is not the same for each individual; for metabolic, environmental or genetic reasons individuals show different responses to nutrients and foods, and, as illustrated in Fig. 1, this is reflected in different level of benefits and risks at the same intake of a

nutrient and different windows of benefit in terms of nutrient intake. Thus, a particular intake of a nutrient may provide risk to some individuals but not others, or a benefit to some individuals and not others. Thus, as shown in Fig. 1, specific nutrient intake based on the whole population may be a risk for some individuals, a benefit for others and have little effect in others.

Genetic factors may be one source of this inter-individual variation. The sequence of the human genome, the increasing amounts of information on individual genetic variation within the human population (single nucleotide polymorphisms; SNP) that is available in databases, and the advent of high-throughput and rapid analytical technologies of SNP analysis has led to the situation where it is possible to address the questions ‘to what extent can we identify sub-groups within populations, and which genetic factors provide them with increased benefit or increased risk from a particular nutrient at a certain intake?’. This has been heralded as an approach that ultimately could lead to ‘personalised nutrition’ where advice on nutrient intake is tailored to specific genetic factors.

There are already a number of companies providing personalised nutritional services. But the questions remain: how effective

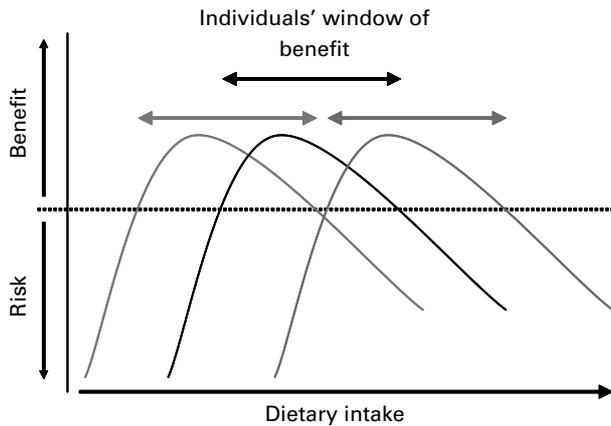


Fig. 1. Illustration of inter-individual variation in response to dietary constituents.

is this approach and what is the future potential of personalised nutrition? There is considerable hyperbole concerning the possibilities that personalised nutrition may offer individuals, much even in the popular press. The reality is that using the results of studies of the influence of genetic make-up (genotype) on response to nutrients may have great potential but much more work needs to be done and the benefits of this research are likely to be a long way off. A substantial research effort is necessary to take advantage of the future possibilities of personalised nutrition: to coordinate and stimulate this, the European Community has funded the European Nutrigenomics Organisation (NuGO) (in 2004), a European network of excellence in the area of nutrigenomics. Nutrigenomics incorporates the use of analytical tools that permit simultaneous analysis of a large number of molecular parameters in nutritional sciences and includes the study of diet–gene interactions and the influence of genotype on response to nutrients, diet and food. To discuss possibilities, hurdles and approaches in this area, a workshop was organised on the role of nutrient–gene interactions in the assessment of the benefit–risk analysis of nutrients and diet. A number of issues of wider nutritional relevance are presented here and are highlighted with examples from presentations at this meeting.

Nutrition–single nucleotide polymorphism interactions

Nutrients can affect biomarkers of benefit or risk. This effect can be influenced by single SNP, small genetic variations between individuals. Thus benefit–risk is not only dependent on the nutrient, but the result of an interaction between nutrient and genotype. That this is of wider human health importance was illustrated by José Ordovás (United States Department of Agriculture Human Nutrition Research Center, Tufts University, Boston, MA, USA), using an example from lipoprotein–cholesterol metabolism. HDL-cholesterol (HDL-C) is a biomarker of susceptibility (or risk factor) to heart disease in current use. Decreased HDL-C is regarded as increasing the chance of heart disease. Increased PUFA intake has been reported to reduce both LDL-C and HDL-C. However, recent work suggests that the response to PUFA depends on genotype. Approximately 75% of individuals are homozygous G (GG) for the apoA1-75(G/A) SNP and

in these individuals increased PUFA intake is associated with decreased HDL-C. However, in heterozygous individuals or AA homozygotes, increased PUFA intake has the opposite effect, increasing HDL-C. This strongly suggests that there is an interaction between diet and genotype in determining the benefit or risk of increased PUFA intake. A specific population group might benefit from a high PUFA diet.

As a single factor, an SNP may have no observable effect on metabolism or phenotype, but it may have under different nutritional conditions. José Ordovás described that the influence of genotype for the $-514(C/T)$ SNP in the promoter of the hepatic lipase gene on plasma parameters of lipid metabolism such as HDL-C are affected by the level of dietary fat. In particular, the T allele is associated with decreased plasma hepatic lipase activity and increased HDL-C, but TT subjects may have impaired adaptation to higher animal-fat diets that could result in higher cardiovascular risk. Similarly, it has been shown that the dietary arachidonic acid intake influences the atherogenic effect of allelic variation in the 5' lipoxygenase gene: the 5' lipoxygenase promoter normally contains five tandem Sp1 binding sites but individuals with variation in the number of these sites show increased atherosclerosis and this effect was enhanced by dietary arachidonic acid but lowered by dietary *n-3* intake; again, this illustrates that nutritional status influences benefit–risk of specific nutrients and genotypes. This was further illustrated by Anne-Marie Minihane (University of Reading, UK) who described how an SNP ($-219G/T$) in the promoter of the apoE gene has little effect on fasting triacylglycerol levels but does influence postprandial triacylglycerol clearance; similarly, following fish-oil supplementation, postprandial triacylglycerol levels were lowered more in the apoE2 subgroup for a specific SNP within the apoE coding region.

Nutrients and multiple single nucleotide polymorphisms

In reality, the situation is even more complex, with many genes and many dietary constituents being involved and analysis of benefit and risk being assessed by a range of different biomarkers. That different SNP interact is illustrated by the following example. Using HDL-C as one phenotypic marker and looking at the apoA1-75 SNP, increased PUFA intake benefits ~25% of the population but the others are at increased risk. Using a second marker, remnant particles and a second SNP (apoA5), José Ordovás argued that increased PUFA intake can have an opposite effect and increase the risk for the majority of individuals. In addition, Anne-Marie Minihane described several SNP that influence postprandial triacylglycerol clearance and she emphasised that for individuals with diabetes or metabolic syndrome, genetic factors influencing postprandial lipidaemia are likely to make a significant contribution to disease pathology and an understanding of diet–genotype–disease relationships would help in the design of strategies to delay disease progression. Thus, the situation is highly complex and we need to have information on SNP in many genes and how these interact with different nutrients before we can definitely conclude what is good for an individual and what is not.

The theme of interaction between multiple SNP was further developed by Iwona Wybranska (Jagiellonian University, Krakow, Poland) in relation to obesity. Obesity is a multifactorial disease with a wide variety of genes being involved. Iwona

Wybranska described studies of the association of genetic variants of a number of candidate genes with obesity. This included leptin, dopamine receptor and melanocortin receptor genes, all of which code for proteins involved in the regulation of food intake, as well as adrenergic receptor and uncoupling proteins which regulate metabolic rate and thermogenesis. Initially the relative risk of SNP in single genes can be determined by the ratio of incidence of disease in groups with different allelic variations. However, interactions between one gene and another, for example, an inverse relationship between SNP in the PPAR- γ and lipoprotein lipase genes that was seen, make benefit–risk assessment more complicated. No clear approach seems to exist on how to deal with this.

Environmental factors

Interactions between different genes and nutrients makes linking genes or nutrients with disease risk complicated. This is even more so since cohorts may differ considerably in nutrition or lifestyle. This was illustrated by using one of the most studied nutrient–gene interactions, namely the relationship between folate intake and the methylene tetrahydrofolate reductase gene. The methylene tetrahydrofolate reductase–C/T677 polymorphism affects enzyme activity, with the T variant resulting in lower activity which can lead to lower plasma folate levels. In turn this may consequently affect plasma homocysteine levels, DNA synthesis and methylation. Several population studies have looked at SNP frequency and folate status in relation to colorectal cancer risk. Ellen Kampman (Wageningen University, The Netherlands) described how these studies have given conflicting results. Several studies, but not all, suggest that the TT genotype for the SNP C/T677 in the methylene tetrahydrofolate reductase gene has an inverse correlation with risk of colon cancer. Differences between countries were seen, which may be due to vitamin B₂ intake. This illustrates the importance of characterisation of the populations under study in the analysis of genetic and nutrient factors in benefit–risk analysis. As pointed out, dissecting out different factors in this benefit–risk equation requires studies with very large cohorts, and generally as one moves to bigger studies the nutritional data are of poorer quality.

Hypothesis-driven candidate gene identification

Both hypothesis-driven and genome-wide screening approaches have been used to identify nutritional relevant SNP that influence phenotype and benefit–risk analysis. The candidate gene approach searches for SNP of potential importance by gene selection on the basis of the physiological roles of the gene products. For example, this ‘hypothesis-led’ approach is being used with selenoprotein genes. Most selenoproteins are known (twenty-five, including Se-dependent enzymes such as glutathione peroxidases and selenoprotein P), which provides a focus for identification of SNP that might influence Se metabolism and nutritional requirements. Catherine Méplan (University of Newcastle, UK) described a strategy to identify key SNP. First, genes or gene regions are identified whose products would be expected to influence Se metabolism (for example, selenoprotein P, selenophosphate synthetase 2, regions of selenoprotein genes coding for 3′ untranslated regions). Second, these genes or gene regions

are amplified in sections of approximately 500 bp and the potential presence of SNP is examined using denaturing HPLC. Regions producing PCR products showing heterozygosity are further investigated by sequencing. Using this approach novel SNP have been identified in selenoprotein P, selenophosphate synthetase 2 and glutathione peroxidase 4 genes and the approximate frequency of different allelic variants characterised in different ethnic groups. The analysis shows some differences between Caucasian, Chinese and Indian groups, demonstrating the influence of ethnicity in SNP studies. The next step is to study the functional significance of these SNP using molecular cell biology approaches, human intervention trials and disease association studies.

Genome-wide screening candidate gene identification

The advent of human genome data and genomic technologies presents an alternative to the hypothesis-led approach in which high-throughput approaches are used to search for genetic variations associated with a particular phenotype or disease. Such an approach has the advantage that the genes one detects as being related to a particular phenotype do not depend upon a hypothesis and therefore one is less likely to miss unexpected genes because one is not looking for them. This approach was described by Martin Wapenaar (University Medical Centre, Utrecht, The Netherlands) using coeliac disease as a model. A search for new genes involved in this disease has been carried out in a sibpair study that used a combination of genome-wide gene association studies and array techniques to identify potential causative genes and disease pathways; having targeted certain chromosomal regions fine mapping was continued by high-throughput SNP studies. However, detection of relevant genes by such approaches has the disadvantage that huge numbers of individuals are needed and thus the studies are very expensive; in addition, SNP that are regulated by diet could be missed because of the difficulty with such approaches of identifying nutrient–gene interactions.

Describing the phenotype

Technically, methods for genotype analysis are very reliable and precise but definition of a phenotype associated with benefit or risk is much more difficult. The healthy phenotype has not been defined and early biomarkers of risk are difficult to define. In any SNP study it is important to be precise as to phenotype, as illustrated by José Ordovás and Anne-Marie Minihane in relation to the question of whether to use fasting or fed blood lipid parameters in SNP studies in relation to heart disease. Similarly, as discussed by Andrew Collins (University of Oslo, Norway), the extent of the individual variation in DNA repair capacity or susceptibility to DNA damage has required careful evaluation, and investigations in this area have been limited by definition of phenotype rather than limitations in knowledge of the key genes. However, there is now good evidence for individual variation in the ability to repair oxidative damage in DNA and that a key gene involved in the repair process, 8-oxoG-DNA glyoxylase, is modulated by nutritional factors. As discussed by Patricia Heavey (University of Ulster, Coleraine, UK), some phytochemicals derived from cruciferous and leguminous vegetables have been reported to be metabolised to compounds

that protect against cancer whilst other carcinogenic food components are thought to be metabolised and detoxified. Individual variation in the ability to metabolise such compounds could give rise to difference between these dietary components influencing susceptibility to cancer. Purely genetic studies do not account for lifestyle or environmental differences; they create a ‘hypothetical individual’ and do not take into account individual responses. Individual responses are of course affected by genetic factors but also by lifestyle, environment and nutrition. It is likely that it is those interactions that are important in determining benefit and risk from nutritional factors.

Probabilistic exposure assessment

To assess whether a risk and benefit occurs it is necessary to know the likelihood of nutritional exposure in relation to a specific genotype. In toxicological exposure studies, probabilistic methods have been introduced that allow determination of the likelihood of exposure. As illustrated by Jacob van Klaveren (RIKILT – Institute of Food Safety, Wageningen, The Netherlands), basically the ranges of exposure (dietary intake) seen in a population and the individuals in the population can be recombined *in silico* and the actual probability of exposure of specific subgroups to specific dietary components can be determined. This provides an approach that is relevant to quantification of the benefit and risk in susceptible population subgroups.

Ethical and cultural issues

Potentially, ethical and societal issues limit the use of genetic studies in assessing benefit–risk and thus the development of ‘personalised nutrition’. Development of genetic, individualised aspects of nutrition will require societal agreement to the use of these technologies. Rein Vos (Maastricht University, The Netherlands) described the main ethical concerns of nutrigenomics as being consent of the individual, privacy and confidentiality of information; this is essentially parallel to pharmacogenetics or genetic testing. In addition, we have to consider to what extent it is ethical to provide foods to combat unhealthy lifestyles. Furthermore, the clear benefit over risk of new products, for example, with increased concentrations, must be evident, especially because exposures are long (over a lifetime).

The gathering and use of genetic and other personal information raises issues, such as the maintenance of databases and the acquisition of samples for analysis. Normally, these issues can be dealt with by informed consent and confidentiality of information about an individual’s personal life. In addition, when optimising advice given to individuals, for example, in the form of personalised nutrition, considerations must be made for differences in nutritional response and thus differences in level of benefit, and especially risk; if the benefit–risk is disease-related the patient–professional relationship must be maintained.

Nutrigenomics will be associated with genetic and technological advancements. Rein Vos identified the relationship between static social norms and dynamic research as a key ethical issue and strongly advocated that the public must be informed and engaged in these developments so that any psychosocial implications can be considered.

In the European context, as pointed out by Nigel Lambert (Institute of Food Research, Norwich, UK), differences between countries in food culture exist and norms and values need to be considered with regard to the ethics and communication of personalised nutrition. It was emphasised that development of genetic, individualised aspects of nutrition will require explanation to the public of what ‘optimal health’ really means.

How to proceed? From single nucleotide polymorphism to nutrient, or nutrient to single nucleotide polymorphism?

The future clearly holds possibilities to use nutrient–gene interaction in assessment of benefit–risk of individuals in relation to requirement and use of specific nutrients or dietary components. However, at present there are a number of important limitations:

It is not possible to define optimal health or to define the key early biomarkers of risk;

In large genetic studies it is difficult to define the critical phenotype;

It is very important to obtain good nutritional and other lifestyle and phenotypic information alongside genetic information, and this often limits very large cohort studies;

Multiple SNP–SNP, SNP–nutrient and nutrient–nutrient interactions lead to a highly complex situation in terms of risk–benefit assessment.

Two broad approaches can be considered. The hypothesis-driven approach starts by identifying a gene product that is affected by diet, then establishing the presence of polymorphisms and identifying their frequency, functional consequences and relevance to human dietary effects. Finally, short- and long-term health benefits or risks of these genetic differences can be assessed based on epidemiological evidence.

The second approach, the genome-wide approach, uses disease or risk marker as a starting point in combination with genome-wide screenings at different levels (transcripts, proteins, metabolites), allowing us to decide which genes to pinpoint, then use SNPchips, denaturing HPLC or similar technology, to identify potentially important polymorphisms. Biobanks, where samples have already been studied for various biomarkers, would be a good source for samples in which to study SNP functionality. Despite the advantage of providing an overview, there are problems with this approach. Whilst genotyping gives genetic information, it gives no clue as to function, and some genetic differences may not be affected by nutrition. In nutrigenomics we need to know particularly about genes that are influenced by diet, and often nutritional data from large disease cohorts are poor. Definition of the healthy phenotype is also imprecise until better biomarkers are found.

The overall consensus was that at present research in nutrigenomics in relation to benefit–risk should focus on the hypothesis-driven, candidate-gene approach. In the first phase there would be identification of genes with SNP, and examination of differences in response to nutrients by individuals. The second phase would then look more closely at the function of the SNP, in relation to other factors including diet, alcohol intake and lifestyle factors. Eventually information concerning several genes and nutrients would be combined and this will be a challenge for bioinformatics.

Conclusions and recommendations

The ultimate goal of nutrigenomics in the area of personalised nutrition should be to provide extra clinical biochemical parameters to assist in advice from medical and paramedical practitioners. Present medical practice is to provide therapies on the basis of diagnosis. Genetics provides a new vision where genetic information could in future be used to provide information on predisposition which together with better early detection will move towards disease prevention. However, such a goal is still far off and much more work is required before we can reliably include genetic factors in any risk–benefit assessment of nutrients and diets.

Acknowledgements

We gratefully acknowledge all speakers at the conference for their contributions and all attendants for their contribution to discussions and the conference atmosphere. However, the views expressed in the present paper are those of the authors and the authors take responsibility for any errors. We kindly acknowledge NuGO, a Network of Excellence funded by the European Union (CT-2004–505944) for funding of the workshop (Nutrition–Genotype Interaction: Benefits and Risks, Wageningen, The Netherlands, 6–7 September 2004; www.nugo.org) and support for writing this report. JH, IW, YD, RE, CP and JK are members of the risk–benefit assessment work package of NuGO.