

## **Nutrigenomics in human nutrition– an overview**

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### **1. Introduction**

The primary aim of this review is to introduce some issues of molecular nutrition to an audience whose majority interest lies elsewhere in the realm of nutrition research. Those within this field can update themselves in recent reviews (Muller & Kersten, 2003; Gillies 2003; Kaput & Rodriguez, 2004). It is wise to start with definitions. The first new term to emerge in this area was “Nutrigenomics” which is unfortunate since the term “molecular nutrition” is more in keeping with established disciplines such as “molecular biology” or “molecular medicine”. Today, “nutrigenomics” is generally taken to refer to the study of how nutrients alter gene expression (Figure 1). The term “nutrigenetics” refers to the study of how normal variation in the sequence of base pairs in a gene alters the extent to which an individual responds to variation in nutrient intake. Nutrigenomics has however also embraced the study of how nutrients influence the consequences of gene expression, namely the synthesis of mRNA (transcriptomics), protein synthesis (proteomics) and metabolite production (metabolomics). The latter three can be combined into a “Nutritional Systems Biology” approach (van Ommen 2004).

Before embarking on an overview of each of these in turn, three important points, each somewhat contentious, can be raised. The first is that the hyperbola which surrounded the advent of the post genomic era has not lived up to its expectation. Sequencing the human genome was first seen as the outer limit of our knowledge of biology but it quickly emerged that knowing the sequence of the genome and understanding how that sequence is regulated are radically different challenges. It is not the genes per se that makes man man and mice mice but rather how the expression of these genes is controlled

(Pennisi 2004). The second issue, which will be raised in subsequent sections with practical examples, is the danger of embracing rapidly advancing technologies by throwing freezers full of plasma samples at the technology in the hope that something beautiful will emerge. Both the hardware technologies and indeed the rapidly advancing software technologies need to be carefully understood for their potential in human nutrition. The final contentious point to be made is that every single major public health nutrition issue of today, has emerged through the successful combination of human nutrition epidemiology and human nutrition intervention studies (cholesterol, folate, homocysteine, long chain n-3 polyunsaturates, fruit and vegetables in certain cancers, foetal programming etc.). This is in absolute contrast with the pharmacological industry where cell and animal models are the starting points of research. This begs the question that what is good and trendy for pharma may not be appropriate for human nutrition. Much of the focus of nutrigenomics today is toward animal and cell models but if it is going to be of significance to human nutrition, then a number of challenges will have to be faced.

## **2. Nutrigenetics**

Human nutrigenetics is a rapidly expanding field and the advent of high throughput genotyping technologies means that many new polymorphic variations on genes will be discovered. There are however two filters which need to be applied to this flow of intervention: How common is the polymorphism and how does it lead to clear metabolic perturbation? Only if the answer is positive to both should we concern ourselves. The huge potential of nutrigenetics to contribute to public health nutrition will be illustrated with some examples

### 2.1 Methyltetrahydrofolate Reduction (MTHFR)

MTHFR is an enzyme involved in homocysteine and folic acid metabolism and a common polymorphism occurs at base pair 677 where thymine (T) replaces cytosine (C). The presence of T allele (either in CT/TC or TT forms) is found at levels of 20% in the Nordic part of Europe, increasing to 40% in the Mediterranean region (Gudnason et al 1998). People who have a version of the gene which contains the C

allele (CC or CT), show no variation in plasma homocystine when folic acid intake is low. However, homocysteine levels more than double when those who are homozygous for the T allele (TT) are put on a low-folate diet (Klerk et al 2002).

## 2.2 Peroxisome proliferator-activated receptor (PPAR )

A polymorphism on the gene for PPAR gamma leads to the exchange of the amino acid alanine for proline (Pro12Ala). Some 26% of the population contain the alanine allele while 74% are homozygous for proline. As the P:S ratio of the diet decrease, mean fasting insulin levels dramatically increase (33 to 46 pmol/l) in plasma with the Alanine allele but not with those homozygous for proline (Luan et al 2001)

Clearly, common genetic variation will come to explain much of the variability we frequently see with dietary therapy. However, two important policy questions arise from this aspect of research. Firstly, do polymorphic changes such as in MTHFR mean that a subset of the population have a higher requirement for a nutrient such that standardised RDA values can no longer operate? Table 1 shows the effect on population RDA if a 25% increase in requirement for a nutrient due to a particular allele occurs at frequencies of zero, 10, 20 and 30% of the population and where the wild type average requirement is 200 with a SD of 15% of that value (Gibney & Gibney 2003). Whilst a 25% increase in requirement seems high, the population impact is relatively small, less than 8% increase. However, alleles will surely be discovered which require less folate for metabolic function and thus things will be evened out in terms of recommended intakes for a population. A second issue concerns the level of evidence which will be needed to accept that a particular polymorphism merits personalised dietary advice. If it is established beyond doubt that a given polymorphism, with a reasonably high frequency, predisposes individuals with the allele to some adverse event under particular nutritional circumstances, then it would be unethical to expose such a patient to such a dietary regimen. The problem is that as yet we do not have any agreed standard by which to evaluate the strength of evidence to make such a judgement.

A final dimension to nutrigenetics is the possibility of going beyond a gene-nutrient interaction for some risk factor for a disease to a multiple gene-nutrient interaction for the disease itself. A major project is underway in the EU ([www.lipgene.tcd.ie](http://www.lipgene.tcd.ie)) to examine a wide range of genes for their interaction with dietary fat intakes in relation to the metabolic syndrome. This involves a prospective cohort study ([www.suvimax.org](http://www.suvimax.org)) of 1,000 cases and 1,000 controls who entered the study in a healthy state and were followed every two years for eight years. The problem which this study and all such studies face is the size of the population. Thus if only 10 genes contribute substantially to population risk and if only two states exist per gene (susceptible variety versus non-susceptible variety), then the number of possible permutations is  $2^{10}$  (1024). Population geneticists continue to call for larger collaborative efforts and that means standardising the collection of data and pooling this data (Khoury, 2004, Khoury et al 2004; Khoury et al 2005). It should however be noted that some success has been achieved in studying the genetic factors influencing plasma HDL cholesterol levels using an approach which examines multiple rare alleles (Cohen et al 2004). Very clearly, the science of nutritional epidemiology will need to re-invent itself, preferably through an international collaborative process.

TABLE 1. Consideration of a hypothetical common polymorphism that, when present, results in a 25% increase in the requirement of a hypothetical nutrient. The calculated increase in requirement for this hypothetical nutrient is presented for allelic frequencies of 0, 10 and 30%, with the final population reference value weighted to take account of these frequencies.

Average requirement (mg.d)	Frequency of allele	Percentage increase in nutrient requirement as a result of allele	sd (%) of requirements	+ 2 SD		
				Original	Allele	Adjusted on a population basis
200	0	-	15	260	-	260
200	10	25	15	260	325	267
200	20	25	15	260	325	273
200	30	25	15	260	325	280

### **3. Nutrigenomics**

The impact of nutrients on gene expression is receiving considerable interest and very significant funding. Many excellent reviews on the topic are available to which the reader is referred (Muller & Kersten 2003). This paper will address a number of the more difficult areas facing nutrigenomics. The technology of cDNA microarrays, using small segments of genes, can process very large numbers of genes to ascertain change in gene expression. However, extensive data exists to show that the choice of propriety technology platform profoundly effects the outcome of the study. In one landmark study, the three leading commercial array platforms were compared for the expression of 150 genes in a PANC-1 cell model exposed under standardised conditions to a standard stimulus. Of the 185 genes only 4 genes showed comparable changes in gene expression (Tan et al 2003). Equally, in a four site comparison of gene expression which confirmed “striking differences” between centres (Waring et al 2004), it was still possible to achieve toxicological classification which could not be done with conventional methods. The scale of change in gene expression in humans in response to diet is unlikely to be as strong as that achieved with the introduction of toxic compounds into animals, and thus the issue of inter-centre and inter-technological platform variability is still a major issue for nutrition. In the US, the National Institute of Standards in Technology ([www.nist.gov/](http://www.nist.gov/)) together with the array technology industry are seeking to find ways of standardising gene array technology. A second challenge which nutrigenomics faces in human nutrition is the limited supply of DNA from different tissues. DNA from peripheral blood mononuclear cells is widely available but it is questionable whether this DNA will reflect the dynamics of DNA in tissues such as the gut, liver, muscle and so forth. Skin, muscle and fat biopsies can be carried out in free-living subjects but these methods are quite invasive and tend to be used only in rare instances. Biopsies of different sections of the gut or liver biopsies are possible but are generally confined to vary rare clinical instances. There is therefore a need to use several animal models to ascertain the relative changes in gene expression in a wide range of tissues in response to

variable nutrient intake to ascertain whether there is any predictive pattern in change. Moreover, these studies should also consider postprandial sampling points particularly if there is any wish to relate changes in gene expression to changes in protein synthesis or metabolite flux. A final issue which will have to be addressed in nutrigenomics is the role of non-coding RNA (ncRNA) in the gene-nutrient interaction. This ncRNA represents a very significant amount of transcribed RNA and to date has been classified as small interfering RNA (siRNA) and microRNA (miRNA) both of which are involved in the regulation of gene expression rather than protein synthesis (Tang 2005).

#### **4. Metabolomics and proteomics**

Relatively few studies relating diet to proteomics are available. Recent work from the Rowett Research Institute (de Roos et al 2005), however, does show the potential of proteomics to reveal hitherto unknown associations between particular proteins and metabolic disorders. In contrast, there is a global surge in optimism for a role of metabolomics in human nutrition (Gibney et al 2005, German et al 2004; Watkins & German 2002). This technology seeks to measure the total or maximal chemical array in biofluids using  $^1\text{H}$ NMR or LC/GC-MS. The enormous signal output from these technologies is then quantified using pattern recognition technology such as principal component analysis or partial least squares discriminant analysis. Metabolomics moves us away from the traditional research approach where an *a priori* hypothesis exists and where there are an array of targeted metabolites to be assayed. Metabolomics allows us to compare different diets and should they exhibit separation using pattern recognition systems, the regions of the NMR or MS spectra which determine dietary differences can be identified. Thereafter, these identified metabolites can be the focus of further studies. Metabolomics has been shown to differentiate controls from disease states including cardiovascular disease (Brindle et al 2002), ovarian cancer (Odunsi et al 2005), hypertension (Brindle et al 2003) and multiple sclerosis ('t Hart et al 2003). The great expectation of metabolomics is that it might offer the prospects of the ultimate biomarker of overall dietary pattern. Theoretically, in monozygotic twins with identical diets and identical microflora, the metabolomic profile or unique metabolic signature should be identical, assuming an absence of disease. On identical diets with identical microflora, in

the absence of disease, metabolomic profiles should vary due to genetic make-up. Preliminary results suggest that dietary patterns can have a powerful effect on metabolomic profiles (Solanky et al 2003; Lenz et al 2004). However, before this technology is applied to human nutrition a number of important areas need to be addressed. By far the most important task is the definition of the human nutrition metabolome, in other words all of the known nutrients, their metabolites and all endogenous metabolites. Standards for these need to be ascertained and metabolomics should be the total capture of all elements of the human nutrition metabolome. For human nutrition this is critically important because human diets contain far more non-nutrients than nutrients, most of which are absorbed, conjugated or otherwise derivitised and excreted into urine or bile. In pharmacology or toxicology studies using animal models this is not usually a problem because the drug or toxin is administered to animals on exactly the same diet. A global initiative on nutritional metabolomics has been initiated by NuGo, the European Nutrigenomics Society ([www.nugo.org](http://www.nugo.org)). The initiative is jointly with the American Society of Nutritional Sciences and the Metabolomics Society and has four working groups : (1) Defining the nutritional metabolome, (2) analysing the human nutritional metabolome, (3) Using the human metabolome to study human nutrition and (4) curating human nutrition metabolomic data with other genomic data or phenotypic data ([www.nugo.org/metabolomics](http://www.nugo.org/metabolomics)). A final and very important area will be studying how the composition of the microflora in the large bowel influences metabolomic profiles (Nicholson et al 2005).

## **5. Nutritional phenotyping**

A recent paper from the American Society of Nutritional Sciences Long Range Planning Committee (Zeisel et al 2005) has raised the issue of defining a nutritional phenotype which would link the “omics” of nutrigenomics to functional and behavioural factors including dietary dimensions. At the end of the day, all of these sciences of the 21<sup>st</sup> century will meet the bottleneck that is a legacy of the last century, which is measuring the human dietary assessment. Two clear weaknesses need to be dealt with. The first is the failure of dietary assessment to deal with meals as opposed to foods and to deal with

meal patterns through some period of time. Without that analytical capability, linking nutrigenomics to public health nutrition will be difficult. The second issue is energy under-reporting and newer ways of looking at food intake data such as probabilistic modelling should help reduce this systematic error in measuring the human phenotype.

## **6. Concluding thoughts**

Some 14% of Utah families have a positive family history of coronary heart disease and these account for 72% of all premature heart attacks (< 50 years) or 48% of heart disease at any age (Hunt et al 2003). This same study also showed that 86% of all Utah strokes are linked to just 11% of Utah families. Clearly, genetics is very important in human disease and is probably far more important than we as nutritionists often care to think. The emerging sciences of nutrigenomics offer great potential to extend our understanding of diet-gene interactions but that requires a multi-disciplinary and international collaborative approach.

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Figure 1. The organogram of nutrigenomics

