Nutrigenomics in perspective

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The sequencing of various mammalian genomes has initiated the development of novel technological applications to study gene expression by high-throughput methods, for as many genes as possible, simultaneously. The merging of these technologies, referred to as transcriptomics, proteomics and metabolomics, with traditional sciences has brought forward new areas of science such as pharmacogenomics, toxicogenomics and nutrigenomics. Nutrigenomics is the monitoring of the interaction of nutrition with gene expression on a genome-wide scale. In recent times it has found its way into all corners of the nutritional field, from crop improvement to microbiotechnology. However, at present the major focus is on trying to describe in molecular terms the influence of diet on human health. Undoubtedly this is due to the awareness that the world is westernizing, and that the modern western diet contributes to the risk of obesity and its complications, such as Type 2 diabetes, cardiovascular disorders and cancer. The growing incidence of obesity among children has raised the alarm that the western world is facing a dramatic increase of patients with nutrition-related diseases with the accompanying healthcare costs. Since diet is part of the cause, the expectations are high that the innovative approaches of nutrigenomics will generate the knowledge that eventually allows the diet to be used as a way to prevent or delay the onset of disease. Thereby, nutrigenomics has found its niche - the pre-symptomatic phase of these disorders [1], when changes in the expression of genes can already be identified. Those changes mark the risk for a next step, that is, the appearance of additional changes in gene expression in connection with overt symptoms. Manipulating the diet would be a way to prevent moving onwards to the second step, and for a large part of the population, this would be considered more acceptable than the preventive taking of drugs.

In its early existence, nutrigenomics faces the challenge of demonstrating its potential, but at the same time encounters many problems, which are only partly specific to nutritional science. Some of those lie in the immaturity of novel technologies. During the past 10 years the use of DNA microarrays for RNA profiling has been tremendously optimized and standardized. Still, the optimal situation does not appear to have been reached, as the use of different platforms may result in (partly) different sets of differential genes. To some extent, this may be due to the different experimental, biostatistical and bioinformatics applications to generate, store, handle and analyze the huge genomics datasets [2,3]. Despite these limitations, transcriptomics by using DNA microarrays is presently the major approach to obtain gene-expression data in nutrigenomics.

At a more functional level, gene expression can be determined by protein profiling. However, the broad range of (bio)chemical properties of proteins hampers the finding of a single technique that can sufficiently cover the complete proteome of a cell, tissue or organism. For nutrigenomics, 2D gel electrophoresis followed by mass spectrometric identification of protein spots is attractive, as this preferentially visualizes the soluble proteins, including many metabolic enzymes. Demonstrating the power of this approach, we have recently studied the temporal changes in molecular processes in the small intestine of calorie-restricted mice: an early down-regulation of energy-consuming processes such as protein synthesis, and at a later stage, the onset of function- and morphology-protecting pathways [4]. To broaden the view on the proteome, liquid chromatographic separation followed by tandem mass spectrometry is becoming increasingly popular. For quantification, labeling methods, such as iTRAC™ and Stable Isotope Labeling with Amino acids in Cell culture (SILAC™) are used, but labeling-free approaches are also being introduced. In the coming years, throughput, sensitivity and...
accuracy of the applied protein-profiling methods will be vastly improved. For nutrigenomics, the wish list includes better ways to readily identify protein isoforms, and better views on the glyco- and phospho-proteome. Nutrition activates molecular pathways, such as the insulin-signaling pathway, which majorly depend on protein modifications. In this respect, the development of protein arrays with antibodies against all isoforms of the components of metabolic pathways is promising. Plasma proteomics by antibody-based multiplex assays is of growing importance for nutrigenomics, and has been addressed by large European projects such as the European Nutrigenomics Organisation (NuGO) [101] and the Diogenes project [102]. Plasma is the medium not only for the transport of nutrients and their derivatives, but also for the nutrient-induced secreted peptides such as cytokines, adipokines and satiety hormones.

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In the area of metabolomics, powerful techniques include nuclear magnetic resonance and gas chromatography–mass spectrometry. However, different classes of nutrients have their characteristic chemical make-up and demand dedicated analytical applications and processing. In addition, not all of the nutrient-related metabolites have been identified and incorporated in the analytical settings. In the short-term, the researcher will likely only obtain information on part of the metabolome. An example is lipidomics for the analysis of the lipid metabolism. One analytical service now provides quantitative data on ten different lipid categories, such as triacylglycerides and phosphatidylcholines, and for each category the constitution by different fatty acids with respect to chain length and degree of saturation is given. Although this provides considerable information, a major additional challenge for nutrigenomics is to determine the dynamic flow of metabolites through organs, such as the lipid handling by the liver and adipose tissue. Questions such as ‘how does the output lipidome of an organ depend on its lipid content and the supply of lipids?’ and ‘how is the output lipidome of the liver reflected in the lipid handling by the adipose or muscle tissue?’ arise. Such ‘fluxomics’ data will become an integral part of metabolomic studies in future nutrigenomics experiments.

A frequent observation in nutrigenomics is that the changes in gene expression induced by changes in nutrition are of relatively low intensity and hard to distinguish from the background variation. This places a high demand on standardization of experimental conditions and requires a higher number of repeat experiments. As a consequence, strategies are designed to generate stronger experimental responses. In fact, the application of drugs in pharmacogenomics does result in much larger changes in gene-expression. Therefore, when a drug interferes with a particular nutrient-dependent molecular pathway, such as rosiglitazone for peroxisome proliferator-activated receptor (PPAR)γ and Wy14643 for PPARα-dependent pathways, such drugs can be used in nutrigenomics experiments as an amplifying replacement for certain nutrients. Other strategies for stronger responses include the use of knock-out and knock-in mice, or interfering RNA inhibition of molecular pathways.

Despite the fact that each discipline is still developing, the combination of omics data is going to provide the ultimate view of gene-nutrient interactions and their effect on health. For instance, by combining transcriptomics and proteomics we demonstrated that insulin-induced adipokine secretion is regulated at the level of post-translational modification by the transcriptional upregulation of protein processing enzymes, whereas secretion was downregulated by rosiglitazone at the transcriptional level [5,6]. Even broader approaches to combine omics datasets into a functional network are being undertaken. Such a network takes all kinds of direct and indirect molecular interactions into consideration and the final overview is referred to as the ‘nutritional interactome’ [7]. Adding time as an extra dimension, the interactome becomes dynamic, and this in combination with other information will eventually create an in silico model of the biological system under the influence of nutrition.

An important concept in nutrigenomics is that nutrients are not only a source of energy or structural components for cells, but have other important functions, such as in the case of antioxidants. A major focus is presently on their function as signaling molecules regulating physiology and metabolism by the regulation of defined target genes. Some nutrients do so by binding as ligands to specific nuclear receptors, and as such interact directly with the promoter of target genes. A classic example is the unsaturated fatty acids which, through their binding to PPARs, can regulate
genes involved in fatty acid oxidation and storage. Similar mechanisms exist for retinoids and amino acids, of which the latter may interact with specific amino acid response element motifs in the promoter region of genes. Such mechanistic knowledge regarding nutrient–gene interactions is regarded as the basis for intervention strategies. The chemical make-up of fatty acids determines their affinity for PPARs. Manipulating the diet with respect to the composition of fatty acids may thus be a way of directing the target molecular processes such that health is sustained or promoted. In general, the mechanistic insight into diet–genome interactions will create opportunities for intervention by dietary advice to the broad public and to particular risk-groups, to which the application of preventive food supplements and functional foods will be advocated by physicians and healthcare workers. Already, cholesterol-lowering dairy foods are recommended to those with a high plasma cholesterol level. These products contain plant sterols that inhibit the uptake of cholesterol from the gut and lead to a reduction of the plasma cholesterol level [8]. For people at risk as determined by biochemical diagnosis, health insurance companies in The Netherlands and Belgium have started to refund the costs of these functional foods. This is likely to become a more general practice, but only after sound scientific proof of the efficacy and safety of the product has been presented. In this respect, nutrients are moving more and more into the medical area as preventive natural agents or ‘nutraceuticals’. However, some nutraceuticals are not without any risk. On the cholesterol-lowering products, a warning has been posted for use by certain persons that may include young children and pregnant/lactating women. This suggests that perhaps the same legislation is going to be applied to nutraceuticals as to pharmaceuticals.

Genetics, epigenetics and sex differences have a significant impact on the future course of nutrigenomics. Response of gene expression to nutrition, functional foods or food toxicity is subject to interindividual genetic variation due to functional polymorphisms in nutrition-related genes. In the past, researchers have tried to find polymorphisms with a significant contribution to health risk. Examples are the folate-related methylenetetrahydrofolate reductase 677C-T and the lipid-related apolipoprotein A5 56C-G polymorphisms with risk for neural tube defects and hypertriglyceridemia, respectively. In theory, in the future such knowledge will allow each individual to be genetically classified according to the risk for certain nutrition-related disorders or food toxicity. Based on this, tailor-made diets and functional foods can be designed to help prevent the risk, a form of personalized nutrition. Some companies have started to incorporate currently known polymorphisms into a DNA diagnostic assay for health. However, this grossly underestimates the complexity of genetics. Single polymorphisms have, in absolute terms, only a very small contribution to the total risk. Therefore, the task is to find most, if not all, of the polymorphisms linked to a trait, study their contributions, alone and in combination with respect to risk, and finally include all of them in a genetic assay to generate individual genetic risk profiles. Of course this is a major challenge, but on the technological level this has become possible [9]. The bottleneck has now moved to data analysis, requesting novel robust applications for multivariate analysis. Recently, the power of such large-scale genome-wide genetic-association studies has been demonstrated by the detection of risk loci together accounting for approximately 70% of the genetic risk for Type 2 diabetes [10]. Nevertheless, the question remains as to whether, in due time, genetic testing will be more efficient than determining gene-expression patterns or metabolite profiles to reveal the individual risk. Familial hypercholesterolemia is due to genetic predisposition with genetic heterogeneity between families. Even if we know all the underlying genetic factors, it would probably be much easier and more uniform to file the diagnosis by simply determining the plasma lipid levels.

Another challenge for nutrigenomics is the so-called Barker hypothesis [11], now more often referred to as the ‘fetal origins’ or ‘fetal programming’ theory. According to this theory, environmental influences on fetal growth and development are reflected in the risk for common disorders with an onset at adulthood. In particular, nutrition during pregnancy seems to play a role. Low birth weight reflecting the poor condition of the fetal stage is found to be associated with increased risk for later-life coronary heart disease, especially in those who had increased weight gain during early childhood [12]. It is thought that the fetal genome can adapt to poor nutritional conditions by epigenetic modification of gene expression via DNA methylation and histone modification [13]. This leads to a thrifty metabolism, which persists postnatally and may lead to obesity and related disorders in later life. This specific area, referred to as ‘nutritional epigenetics', is
In nutrigenomics, as in pharmacological sciences, sex and gender differences require much more attention than they usually receive. Significant sexual dimorphic gene expression, as determined by DNA microarrays in the mouse on a Western diet, was found in liver, adipose, muscle and brain for 72, 68, 55 and 14% of active genes, respectively [14]. In addition, sex differences have been observed in many genetic-association studies, and seem to be a common phenomenon. Apparently, sex differences should be an integral aspect of the development of functional foods. In fact, this is not something new, but is extending on an existing practice: for example, calcium for postmenopausal women, zinc to improve prostatic function in men and folate for preconceptional women.

Summarizing the above, we can conclude that nutrigenomics is an established discipline, but is, however, still in its childhood and has to grow to its full potential with respect to the various areas of nutritional science and food biotechnology. Since research and development in nutrigenomics are complex and expensive, the best way to progress is by forming close collaborations between the various nutritional research institutes and food industries. The NuGO network of excellence is one such existing initiative, and hopefully this can be expanded to a worldwide scale [101].

Bibliography


Websites

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102. Diogenes project www.diogenes-eu.org