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Nutrigenomics in the modern era

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The concept that interactions between nutrition and genetics determine phenotype was established by Garrod at the beginning of the 20th century through his ground-breaking work on inborn errors of metabolism. A century later, the science and technologies involved in sequencing of the human genome stimulated development of the scientific discipline which we now recognise as nutritional genomics (nutrigenomics). Much of the early hype around possible applications of this new science was unhelpful and raised expectations, which have not been realised as quickly as some would have hoped. However, major advances have been made in quantifying the contribution of genetic variation to a wide range of phenotypes and it is now clear that for nutrition-related phenotypes, such as obesity and common complex diseases, the genetic contribution made by SNP alone is often modest. There is much scope for innovative research to understand the roles of less well explored types of genomic structural variation, e.g. copy number variants, and of interactions between genotype and dietary factors, in phenotype determination. New tools and models, including stem cell-based approaches and genome editing, have huge potential to transform mechanistic nutrition research. Finally, the application of nutrigenomics research offers substantial potential to improve public health e.g. through the use of metabolomics approaches to identify novel biomarkers of food intake, which will lead to more objective and robust measures of dietary exposure. In addition, nutrigenomics may have applications in the development of personalised nutrition interventions, which may facilitate larger, more appropriate and sustained changes in eating (and other lifestyle) behaviours and help to reduce health inequalities.

Nutrigenomics: Nutrigenetics: Metabolomics: Microbiome: Personalised nutrition: Organoids: Genome editing

On 13 December 1902, Archibald E. Garrod published a landmark paper in *The Lancet* in which he described his observations of individuals with alkaptonuria, also known as black urine or black bone disease[1]. By careful investigation of the histories of thirty-two known examples, he noted that nineteen occurred in seven families and that the condition was more common in offspring from marriages between first cousins[1]. This helped to establish the genetic basis of the condition, but more than 90 years elapsed before a Spanish team established that mutations in the *HGD* gene, which encodes the enzyme homogentisate 1,2 dioxygenase are causal for the disease[2]. Garrod's research led to the realisation that the laws of Mendelian inheritance apply to *Homo sapiens* (alkaptonuria is a classical example of an autosomal recessive disease) and initiated the study of inborn errors of metabolism, which we now know are individually rare but collectively common. Garrod's paper was subtitled 'A study in chemical individuality' and reflected his discovery that human biological individuality can be manifested as differences in the chemistry of body fluids[1], what we would now describe as the metabolome. He wrote '. . .just as no two individuals of a species are absolutely identical in bodily structure neither are their

Abbreviations: CNV, copy number variation; CRISPR, clustered, regularly interspaced, short palindromic repeats; PN, personalised nutrition; Se, selenium.

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chemical processes carried out on exactly the same lines...'[1].

Almost 60 years later, Dr JA Roper from the University of Glasgow presented the first paper at a Nutrition Society meeting on the links between nutrition and genetics entitled 'Genetic determination of nutritional requirements'[3]. Focussing largely on what had been learned from studies in bacteria, Roper concluded that studies in microorganisms had brought '...a clearer understanding of the relationship of genotype and nutritional requirements' and that this had '...opened a new approach and a new way of thought to a large aspect of genotype-environment interaction'[3].

Despite this insight and foundation in basic science, there was relatively little progress in understanding genotype–nutrition interactions in human subjects until initiation of the Human Genome Project, which was launched in 1990. On 26 June 2000, with a great deal of razzmatazz at a joint trans-Atlantic press conference, Mr Bill Clinton and Mr Tony Blair announced that the first draft of the human genome had been completed. This ushered in the 'big science' era in biology and led to extravagant claims about the ways in which outcomes from genomics research would revolutionise diagnosis, treatment and prevention of disease. Within a year, Peregrin predicted that the future of nutrition would be 'nutrigenomics' i.e. study of the interactions between genes and nutrition[4]. Throughout the first decade of this century, the science of nutrigenomics developed rapidly, stimulated by major infrastructure projects including the European Nutrigenomics Organisation[5] and by increasing access to concepts, tools and resources from other areas of biology.

For the purposes of this review, I will consider nutritional genomics (or nutrigenomics) as that branch of science concerned with all types of interactions between nutrition and the genome and which is characterised by the application of high-throughput genomic (or genome-related) tools. Nutritional genetics (or nutrigenetics) is a sub-set of nutrigenomics, which focuses on understanding how genomic variants interact with dietary factors and the implications of such interactions.

Nutrigenetics: impact of genetic variation

Early tests of Garrod's prediction that different genetic variants could lead to different phenotypes involved studies of candidate gene variants (usually SNP) in small numbers of people who might not be representative of the target population. Unsurprisingly, the findings from such studies could be difficult to replicate and were likely to be subject to publication bias because researchers and journals were more likely to publish 'positive' findings. This area of science was transformed by the availability of tools, which enabled very large numbers of genetic variants to be interrogated simultaneously in large population groups. What is now known as genome-wide association studies is a very powerful approach for investigating genotype–phenotype relationships and for example, was used to discover the role of *FTO* gene variants in adiposity[6]. The mechanism through which altered *FTO* genotype influences adiposity is unclear. An early study of ninety seven Scottish children suggested that the *FTO* risk variant predisposes to obesity through hyperphagia or a preference for energy-dense foods[7]. However, a recent systematic review and meta-analysis of fifty-six studies reporting on 213 173 adults found that the *FTO* risk allele is associated with lower (not higher) reported total energy intake and with altered patterns of macronutrient intake[8]. Although statistically significant, these differences were small and it is uncertain whether the associations are independent of dietary misreporting[8].

Unlike the 'simple' Mendelian genetics, which is responsible for inborn errors of metabolism such as alkaptonuria or phenylketonuria, obesity and other diet-related diseases are seldom due to single genetic variants. Indeed, recent analyses suggest that at least ninety-seven variants are involved in body fatness (Fig. 1a)[10] and together these explain <3 % of the variance in BMI[9]. The affected genes are involved in multiple pathways within the central nervous system (regulating e.g. food intake and/or satiety) and in aspects of metabolism including lipid metabolism and adipogenesis (Fig. 1b)[10]. In addition, variants in genes involved in cell biology and cell signalling and in RNA binding/processing are also associated with adiposity risk (Fig. 1b)[10]. Variants in forty-nine loci are associated with differences in the distribution of body fat[11] with high BMI/low waist : hip ratio and high BMI/high waist : hip ratio giving the classical pear and apple shapes, respectively (Fig. 1a)[10]. These fat distribution-related genetic variants are found in genes involved in adipogenesis, angiogenesis and regulation of transcription (Fig. 1b)[10] but how they determine differences in patterns of body fat accumulation (body sites where adipocytes expand preferentially) remains to be elucidated. Information on such genetic variants can be used to develop genetic risk scores[12], which may provide a valuable overall measure of the genetic component of obesity risk for use in diet–gene interaction studies.

The early phase of nutrigenetics research was characterised by studies of candidate gene variants in small numbers of participants, often with relatively crude characterisation of dietary intake or nutritional exposure. Given that lifestyle factors such as nutrition play a major role in the aetiology of most common complex diseases[13], it took a surprisingly long time for those using genome-wide association studies approaches to begin to consider how dietary (and other environmental factors) might interact with genotype to modulate human phenotypes[14]. There are now several studies which confirm that the adiposity risk associated with the *FTO* gene is attenuated by higher physical activity[15,16]. In addition, investigations using the outcomes from genome-wide association studies demonstrate that dietary factors interact with genotype to determine adiposity. For example, Qi *et al.* showed that intake of sugar-sweetened beverages interacts with genetic risk of obesity to amply inter-individual differences in BMI[17]. Similar interactions were reported for fried foods[18]. Dietary patterns may also interact with genetic risk score to determine adiposity related outcomes[19].

Mapping of the first human genome, announced by Clinton and Blair in 2000, had taken 10 years work by

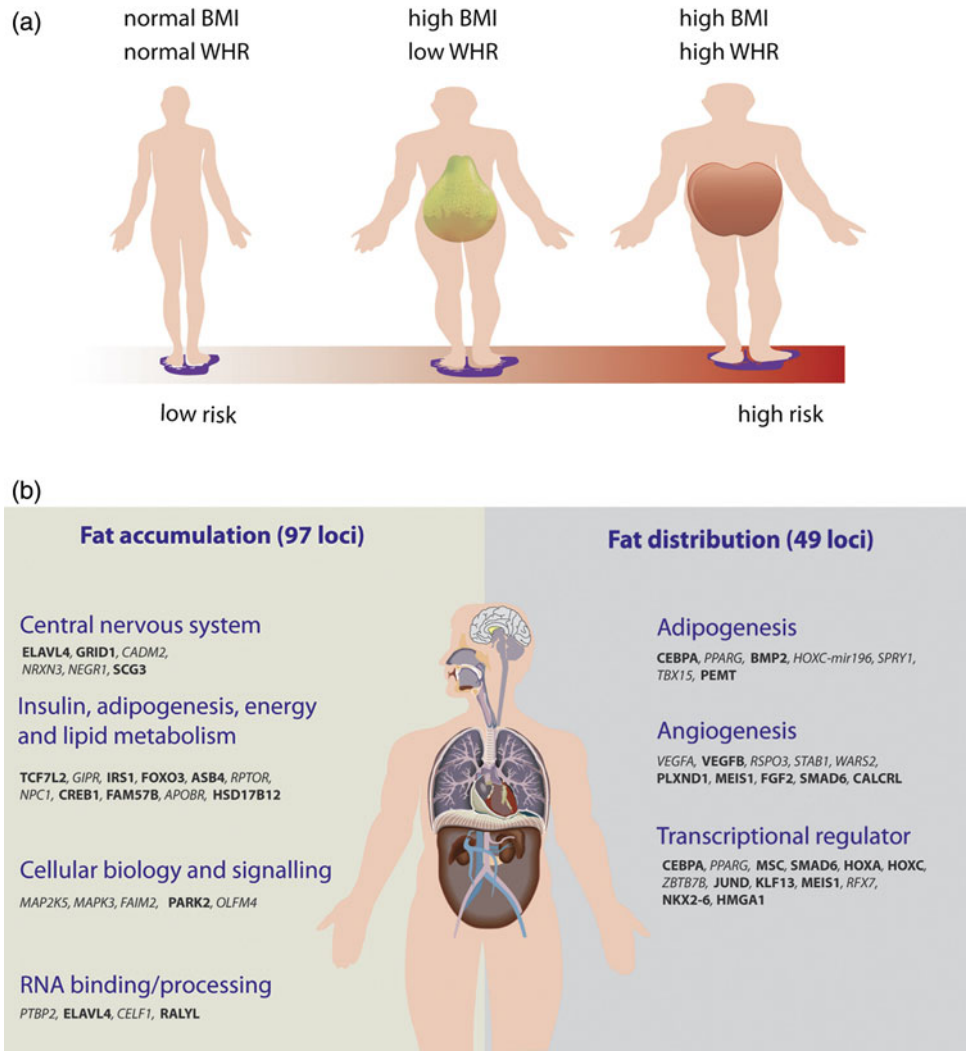

Fig. 1. (Colour online) Genetic contribution to human adiposity (figure from Fu *et al.*[10]). (a) Human body shapes caused by different amounts and different distribution of body fat. (b) Examples of genetic variants, pathways and processes associated with fat accumulation and fat distribution. WHR, waist:hip ratio.

international teams of researchers and cost >$100 million. Very rapid advances in the technologies for direct sequencing of DNA have meant that the cost and time required to obtain the complete sequence of a human genome have fallen dramatically. By 2013, it was possible to sequence a human genome for about $5000[20] and in 2016 this can be achieved by a robot within 24 h for < $1000. Such advances have facilitated the analysis and cataloguing of sequence variation in very large numbers of individuals and, for example, the Exome Aggregation Consortium announced recently details of sequence variation of all protein-coding regions (exomes) of 60 706 individuals of diverse ancestries[21].

Copy number variants

Investigation of human genomes has revealed several different types of genomic structural variation[22]. In addition to the widely-studied SNP, such variation includes gene deletion, inverted gene sequences, copy number variation (CNV; multiple copies of the same gene) and segmental duplication (duplication of a larger segment of the genome containing two or more genes)[22]. CNV are very common, are found on all chromosomes and depending on the level of stringency applied in their definition, account for 4·8–9·5 % of the human genome[23].

CVN affect multiple cellular processes and the earliest discovery of a nutrition-related CNV was in *AMY1*, the gene encoding salivary amylase[24]. *AMY1* CNV are functionally important because *AMY1* copy number is correlated positively with salivary amylase protein concentration[24]. Furthermore, Perry *et al.* argued that diet may have been a factor influencing natural selection of CNV in *AMY1* because individuals from populations with high-starch diets have, on average, more *AMY1* copies than those with traditionally low-starch diets[24]. In independent studies, increased *AMY1* copy number was positively associated with amylase gene expression

and serum enzyme concentration and perhaps surprisingly, reduced *AMY1* copy number was associated with increased BMI and increased risk of obesity[25]. This association between higher copy number for *AMY1* and lower obesity risk was confirmed in a study of Mexican children[26]. The human genes encoding salivary amylase (*AMY1*) and pancreatic amylase (*AMY2*) have very high-sequence identity and are found close together on chromosome 1[27]. Although Falchi *et al.* reported no, or only weak, associations between *AMY2* copy number and BMI[25], Carpenter *et al.* argued that technical limitations in the approach used by Falchi *et al.* and the correlation in copy number between *AMY1* and *AMY2* mean that the specific link between amylase gene CNV and adiposity remains uncertain[27].

Although CNV in at least eighty-four genomic loci have been associated with human adiposity, of these, only four have been associated with BMI or obesity in multiple studies[28]. In an analysis of 1850 European-Americans and 498 African-Americans, including CNV in genetic models of BMI category risk did not add significantly to the variation explained by SNP[28]. In contrast, a study of Chinese children showed that higher genetic risk score, based on CNV at three loci (10q11·22, 4q25 and 11q11), was associated with higher risk of obesity[29]. The latter study also showed that dietary preferences, e.g. a meat-dominant diet, may interact with the CNV at 10q11·22 to increase obesity risk[29].

Nutrigenomics: understanding mechanisms

In a review of the emerging science of nutrigenomics, Michael Muller and Sander Kersten predicted that '… applied wisely, it will promote an increased understanding of how nutrition influences metabolic pathways and homeostatic control, how this regulation is disturbed in the early phase of a diet-related disease and to what extent individual sensitizing genotypes contribute to such diseases'[30]. This aim of increasing understanding of the molecular actions of nutrients and other dietary components and of their roles in the maintenance of normal and disturbed cellular homeostasis remains a central ambition of nutrigenomics. Many nutrigenomics investigations involve largely hypothesis-free study designs in which differences between two or more conditions (e.g. dietary treatments) are explored using genome-wide analyses at the transcriptome, proteome, metabolome and/or epigenome levels. Then, using sophisticated bioinformatics tools, attempts are made to identify genes, pathways and processes, which differ between the conditions and to use this information to illuminate the mechanisms responsible for these different conditions. Such mechanistic studies are often carried out in experimental animals, e.g. the nematode *Caenorhabditis elegans* or mice, because of the potential for greater control over confounding variables. For example, Lange *et al.* used genome wide transcriptomic approaches to investigate the effects of five types of dietary fibre on gene expression in the murine colonic mucosa[31]. They observed that resistant starch (which was not completely fermented in the colon) evoked a distinctive transcriptional response whilst expression profiles responses to the other four fibre sources were similar[31]. Importantly this study identified the nuclear receptor PPAR-γ (a member of a superfamily of ligand-inducible transcription factors) as an important regulator of the gene expression responses in the colonic mucosa[31].

Classical meal feeding paradigms have been used extensively in nutrigenomic investigations. For example, in the fasted state, twenty-one healthy young men were given shakes containing predominately SFA or PUFA and post-prandial genome-wide gene expression changes in peripheral blood mononuclear cells were quantified using a microarray approach[32]. Ingestion of PUFA decreased, whilst SFA intake increased, expression of genes involved in liver X receptor signalling and PUFA also increased expression of genes related to cellular stress responses[32]. Such findings may help to explain the differential effects of these dietary lipid classes on metabolic processes and, ultimately, on health outcomes.

Nutrigenomics studies may also attempt to integrate data from different omics approaches to provide a more holistic understanding of an issue. Recently, we embedded such a nutrigenomics investigation within the Biomarkers of Risk in Colon Cancer study[33]. Since low selenium (Se) status is associated with higher risk of large bowel cancer, we tested the hypothesis that adverse effects of low Se would be evident in the apparently-normal colorectal mucosa[34]. We used both proteomic and transcriptomics approaches to investigate gene expression in colorectal mucosal biopsies from twenty-two healthy adults who were discordant for plasma Se concentration but matched for BMI, age and sex[34]. Integrating proteomics and transcriptomics datasets revealed that the low Se group had reduced inflammatory and immune responses and cytoskeleton remodelling, which suggests that suboptimal Se status may reduce cellular capacity to respond to inflammatory and oxidative stresses and, therefore, increase bowel cancer risk[34].

Nutrition and the gut microbiome

Over the past decade, there has been an explosion of interest in the impact on health of microbes in, and on, the human body. It is becoming apparent that there are complex interactions between the microbiome, the immune system and whole body metabolism and that dietary factors may be central to, and may modulate, many of these interactions (Fig. 2)[35]. Our largest, most dense ($>10^{11}$ cells per g contents) and metabolically active microbial community is in the large bowel and this flora is dominated by anaerobic bacteria belonging to two phyla, the Firmicutes and Bacteroidetes[36]. Unsurprisingly, the composition and metabolic activity of the large bowel microflora is strongly influenced by food intake as reported in both observational[37] and intervention[38] studies. However, establishment of causal relationships between the human gut microflora and health outcomes such as obesity[39] or cancer[36] is more challenging.

Recent research has shown that maternal malnutrition is associated with major changes in the infant gut

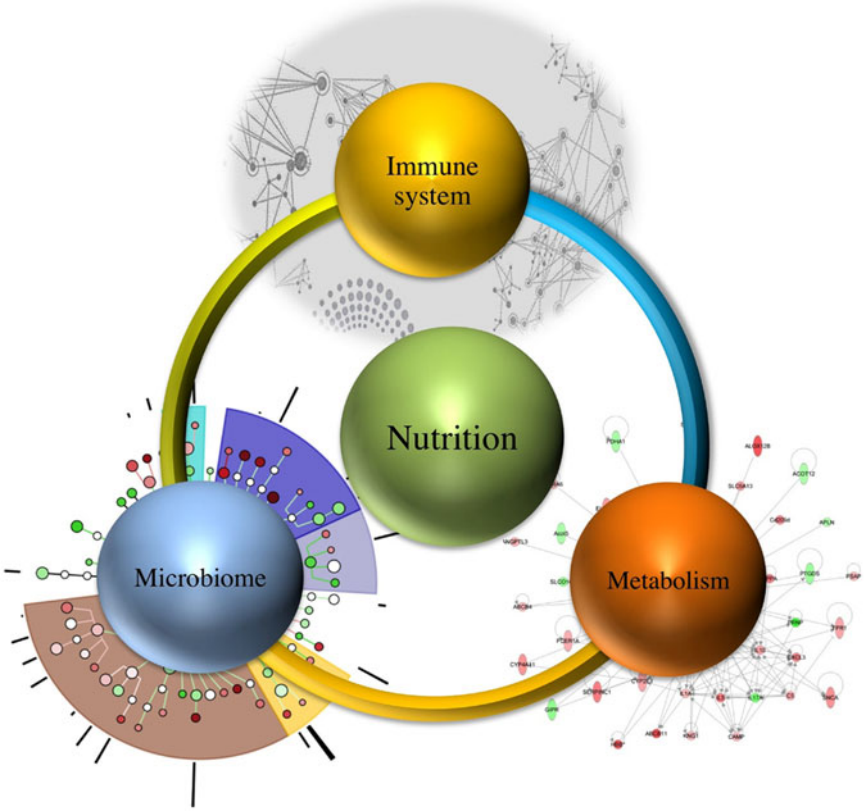

Fig. 2. (Colour online) Schematic representation of interactions between nutrition, the micriobiome, the immune system and metabolism (figure from Verma *et al.*[35]).

microbiome and that these effects may be mediated by sialylated breast-milk oligosaccharides[40]. These sialylated breast-milk oligosaccharides (and, perhaps other milk-derived components, which escape small bowel digestion) induce transcriptional responses in particular bacterial species, e.g. *Bacteroides fragilis,* which initiate a 'cross-feeding' cascade affecting other members of the microbiota (Fig. 3)[41]. Encouragingly, in animal models (mice and piglets), the addition of sialylated breast-milk oligosaccharides improved growth and development[40] but translational benefits in human infants remain to be demonstrated.

Nutrigenomics: applications to improve public health

Novel biomarkers of dietary intake

A major impediment in applying the outcomes from nutrition research to improve public health is the limitation in methodologies available for assessing dietary intake[42]. Here nutrigenomics approaches may advance the field through the application of metabolomics to discover novel biomarkers of food intake[43]. This approach is predicated on the concept that individual foods contain large numbers of (often) unique metabolites (food metabolome), which, after digestion, absorption and further metabolic processing give rise to characteristic metabolites in body fluids including blood, saliva and urine (Fig. 4)[43,44]. Such metabolites can be detected using both targeted and untargeted metabolomics approaches. However, most biomarker discovery work to date has employed untargeted approaches and NMR and/or chromatography prefaced MS-based technologies[43,44]. Given the chemical complexity of the metabolites in foods and the multiplicity of changes induced during digestion and metabolism, identification of the specific food-derived metabolites present in body fluids remains challenging[44]. We have developed a standardised test meal-based protocol[45] which has proved suitable for detection of a number of novel urinary biomarkers of food intake[46–48]. Examples of putative biomarkers of specific foods identified through the use of untargeted metabolomics approaches are shown in Table 1. Foods such as sucrose, which are essentially single molecules and that, after digestion, give rise to metabolites that are difficult to distinguish from endogenous metabolites, pose particular challenges in using metabolomics approaches[49].

Given the speed of progress in using metabolomics to discover and validate novel biomarkers of food intake, which can be detected and quantified in body fluids such as urine, blood and saliva, it seems probable that we will be able to use these new biomarkers to obtain comprehensive and objective information on dietary exposure. This has potential to revolutionise dietary assessment by reducing the cost of making the measurements whilst simultaneously increasing the quality and

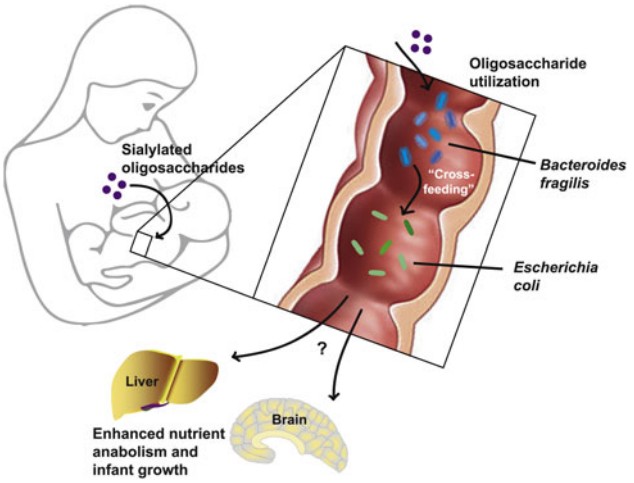

Fig. 3. (Colour online) Sialylated oligosaccharides in the breastmilk from well-nourished mothers are substrate for gut bacterial species such as *Bacteroides fragilis* and support a cross-feeding cascade with other species. This may promote growth and development of the infant through provision of bacterially-derived metabolites and other factors (figure from Bashiardes *et al.*[41]).

reliability of the collected data. This will then shift the research focus to the development of easy-to-use technologies for self-collection of urine (and possibly other body fluids) at home to facilitate repeat assessments of dietary exposure and so provide a better measure of habitual dietary intake.

Personalised nutrition to enhance behaviour change

At the level of the individual, improving nutrition focuses on changing behaviours: what, when, where, with whom and how much to eat. Conventional approaches to achieving dietary change often use a one-size-fits-all approach e.g. 'eat at least 5 portions of fruits and vegetables daily'. Such approaches can be effective but often result in only modest improvements in food intake[50]. A personalised nutrition (PN) approach is based on the hypothesis that knowledge of key characteristics of those to whom the intervention is being delivered will help in making the intervention more relevant and may increase motivation to make, and to sustain, the desired dietary change[51]. Given the importance of interactions between diet and genetics in determining health, the use of genotypic information in designing and delivering personalised dietary advice has been a goal since the beginning of the modern nutrigenomics era[4,52].

In a study involving 138 healthy young men and women, Nielsen & El-Sohemy tested the idea that provision of genotype-based dietary advice would result in bigger changes in dietary behaviour than advice to follow the generic Canadian dietary guidelines[53]. At 12 months follow up, those participants who had been informed that they carried the risk allele of the *ACE* gene and, therefore, should limit their sodium intake reported greater reductions in salt intake than the control group (generic dietary advice)[53]. However, there were no differences in intake for any of the other three targeted dietary components i.e. caffeine, vitamin C and added sugars[53].

The Food4Me PN intervention study was a Europe-wide randomised controlled trial, which tested the hypothesis that providing personalised dietary advice will improve dietary intakes and markers of health, including body weight and waist circumference[54]. Adults (1607) from seven European countries were randomised to: (i) conventional dietary advice (Control) or to PN advice based on: (ii) individual baseline diet; (iii) individual baseline diet plus phenotype (anthropometry and blood biomarkers); or (iv) individual baseline diet plus phenotype plus genotype (five diet-responsive genetic variants)[54]. Participants in the study were recruited via the internet, collected and uploaded data via the web and collected biological samples (blood and buccal cells) at home using kits sent by post from the research team. In addition, they received dietary advice and other information from the research team by email and via the web[54]. At 6 months follow-up, participants randomised to the PN treatments showed significantly greater improvements in dietary intake, consumed less red meat, salt and saturated fat, and increased folate intake and had higher Healthy Eating Index scores than those randomised to the Control arm[55]. There was no evidence that including phenotypic or phenotypic plus genotypic information enhanced the effectiveness of the PN advice[55]. The Food4Me PN randomised controlled trial demonstrates that a personalised approach can produce greater improvements in eating behaviour than conventional one-size-fits-all approaches. However, at least in the context of this study, the nature of the information used to personalise that advice did not affect the outcome.

The Food4Me PN randomised controlled trial showed that the internet can be used to collect information on relevant participant characteristics and to deliver PN to large numbers of people so that this approach is potentially scalable. This could be a significant advance on conventional face-to-face interventions where the resource implications of collecting and processing the relevant information could mean that such PN interventions would be limited to more affluent sections of society. It is important that interventions should aim to narrow, rather than to exacerbate, health inequalities. In that context, digital-based technologies for intervention delivery may offer several potential advantages including convenience, scalability and reduced costs and so have the potential to narrow health inequalities[51]. In addition, in the Food4Me PN randomised controlled trial, we observed that the quality of anthropometric data collected and recorded by participants themselves was high[56] and that participants could use dried blood spot cards at home to collect blood for nutrient status and metabolite measurements[57]. These technical innovations offer considerable promise not only for future translational research aimed at developing and implementing PN interventions but also in conducting a wide range of nutrition research under real world conditions.

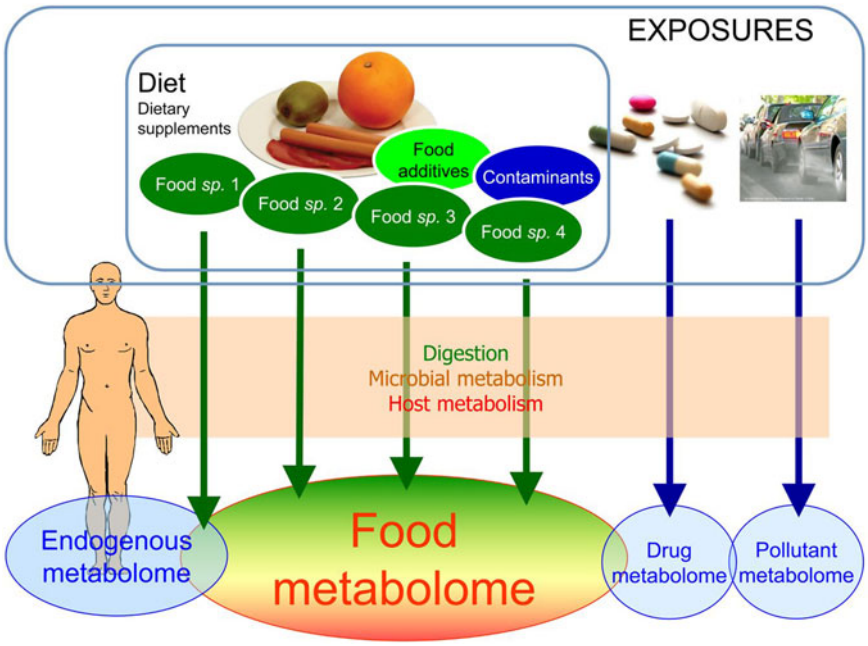

Fig. 4. (Colour online) The metabolite composition of body fluids including urine, blood and saliva reflects the metabolite composition of consumed foods after digestion and metabolism. This food metabolome can be used to identify biomarkers of intake of individual foods and of eating patterns (figure from Scalbert *et al.*[44]).

Table 1. Examples of putative novel biomarkers of intakes of specific foods identified using untargeted metabolomics approaches*

Food	Putative biomarker
Citrus fruit/ juice	Proline betaine
Raspberries	Caffeic acid-sulphate, methylepicatechin-sulphate
Cruciferous vegetables	S-methyl-L-cysteine sulphoxide, sulphoraphane N-acetylcysteine
Wholegrain rye bread	3,5-dihydroxycinnamic acid-sulphate
Red meat	O-acetylcarnitines
Salmon	Anserine, methylhistidine, trimethylamine-N-oxide
Coffee	N-methylpyridinium, trigonelline, dihydrocaffeic acid
Chocolate	6-amino-5-(N-methylformylamino)-1-methyluracil, theobromine, 7-methyl-uric acid
Walnuts	5-hydroxyindole-3-acetic-acid

* Data from Scalbert *et al.*[44].

New opportunities for nutrigenomics

Mini-guts and other organoids

Better models facilitate better hypothesis testing and promote better science[58]. Recent developments in stem cell biology offer exciting opportunities for developing new models for use in nutrigenomics research. Stem cells are undifferentiated cells within multi-cellular organisms, which are capable of unlimited replication and whose progeny can differentiate into several different cell types[59]. For example, hematopoietic stem cells within bone marrow give rise to all types of blood cells including myeloid (e.g. macrophages, erythrocytes and platelets) and lymphoid (e.g. T cells, B cells and natural killer cells) cell lineages. Research over the past three decades has resulted in the development of appropriate culture conditions and protocols for the generation of many cell lineages from both embryonic[60] and adult stem cells[61]. In addition, the Nobel Prize winning discovery[62] that differentiated cells such as fibroblasts can be reprogrammed to an embryonic-like state to produce what are known as induced pluripotent stem cells[63] produced a step change in understanding of the regulation of cell differentiation and laid the foundation for the new translational science of regenerative medicine.

Nutrigenomic research in individual adult human cell types is no longer restricted to using tumour-derived or transformed cell lines and can employ specific cell types derived by differentiating embryonic stems cells and induced pluripotent stem cells. Potentially even more exciting is the use of stem cells to derive three-dimensional cultures of organoids[64]. When grown under appropriate culture conditions, cell progeny from embryonic stems cells or induced pluripotent stem cells self-organise into three-dimensional organoids, which are small versions of their *in vivo* counter-parts[64]. Clevers and coauthors were the first to demonstrate that appropriate culture of single stem cells from the small intestine (identified by the gut epithelial stem cell marker Lgr5) would self-organise to produce crypt-villus organoids containing multiple differentiated cell types[65]. Similarly, single $Lgr5^{+ve}$ cells from the stomach could be cultured *in vitro* to generate efficiently long-lived organoids resembling the mature pyloric epithelium[66]. This approach

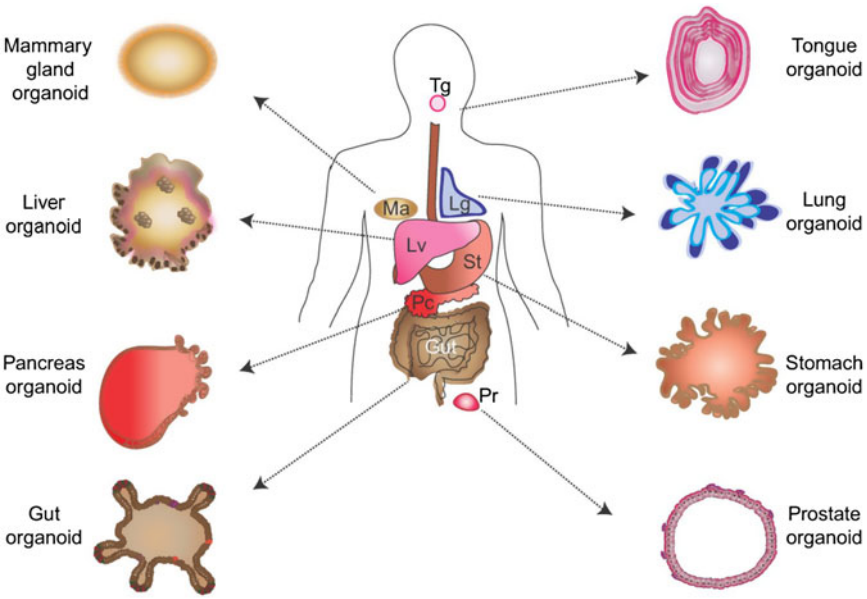

Fig. 5. (Colour online) Organoids can be grown starting from stem cells derived from multiple adult human tissues. These provide excellent, tractable models for study of the effects of nutrition on growth and development in health and in disease [figure from Huch & Koo[64]].

has now been used to derive organoids from multiple organs and tissues including mammary gland, liver, pancreas and lung, which recapitulate key features of the corresponding human organs (Fig. 5)[64]. Human cerebral organoids, derived from pluripotent stem cells, which develop various discrete, although inter-dependent, brain regions have also been described[67]. Such organoids are likely to be better models of the complexity of the *in vivo* situation and their use provides nutrigenomics researchers with huge opportunities to undertake well-controlled experiments using very tractable models to investigate the mechanisms through which food components modulate development and function in both health and disease.

Genome editing

Reductionist, mechanistic research on diet–gene interactions has been accelerated by the ability to target specific genes using gene knock out and knock in approaches. Until recently, such approaches have been limited by side effects (off-target effects), which may not yield the desired phenotype. However, this field is being revolutionised by the development of much more specific genome editing techniques including use of zinc finger nucleases, transcription activator-like effector nucleases and clustered, regularly interspaced, short palindromic repeats (CRISPR)[68]. In particular CRISPR, which uses short RNA sequences to target specific genomic locations, together with CRISPR-associated (Cas) enzymes can be highly specific in directing DNA cleavage[68]. Another advantage of the CRISPR-Cas approach is that it can be multiplexed to facilitate the deletion and/ or insertion of multiple genomic domains. Proof of principle of the utility of CRISPR-Cas in primates was demonstrated first by a Chinese team at Nanjing Medical University who reported the birth of two macaques (Ningning and Mingming) in which the *PPAR-γ* and *RAG-1* genes had been knocked out using the CRISPR/Cas9 system at the one-cell embryo stage[69]. Importantly, this simultaneous disruption of two target genes was achieved in one step with no evidence of off-target mutagenesis[69].

The use of genome editing approaches such as CRISPR-Cas to target specific genes or processes in stem cell-derived organoids provides biologists, including nutrition scientists, with unparalleled opportunities for innovative, mechanistic research. Because organoids can be derived from adult stem cells[64], this approach can begin to elucidate the mechanisms responsible for inter-individual differences in metabolism or other functions and the potential impact of nutritional factors. In principle, genome editing techniques could be used to correct nutrition-related inborn errors of metabolism such as alkaptonuria, phenylketonuria or cystic fibrosis[64]. Using such approaches in somatic tissues would have parallels with applications in regenerative medicine and are likely to be relatively non-controversial. However, the potential to use genome editing to 'correct' germ-line defects is much more revolutionary and raises significant ethical questions[70]. On 1 February 2016, researchers at the Francis Crick Institute, London received permission from the UK Human Fertilisation and Embryology Authority to use CRISPR–Cas9 technology in human embryos for early-development research[71]. This was the first endorsement of such research by a national regulatory authority and is aimed at new treatments for infertility. In this example, the modified embryos will be destroyed after 7 d but there is speculation that it may not be long before

the first CRISPR-modified human baby is born elsewhere in the world[71].

Conclusions

Nutrigenomics is a young science, which is developing well. Much of the earlier hype around possible applications of the science was unhelpful and raised expectations which have not been realised as quickly as some would have hoped. However, major advances have been made in quantifying the contribution of genetic variation to a wide range of phenotypes and it is now clear that for nutrition-related phenotypes, such as obesity and common complex diseases, the genetic contribution provided by SNP alone is often modest. There is still much to be done to understand the roles of less well explored types of genomic structural variation, e.g. CNV, and of interactions between genotype and dietary factors in phenotype determination. Metagenomics-based studies suggest that the gut (and other) microbiota may have unexpected, and substantial, effects on multiple aspects of human health and that nutrition may be the most important mediator of the composition and function of our commensal flora. New tools, including stem cell-based approaches and genome editing, have huge potential to transform mechanistic nutrition research. Whilst recognising that some of these technologies bring with them significant ethical questions, nutrition researchers should grasp the opportunity to address questions of how nutrition modulates function with implications for health at all stages of the life-course. Finally, the application of nutrigenomics research offers substantial potential to improve public health e.g. through the use of metabolomics approaches to identify novel biomarkers of food intake and of dietary patterns, which will lead to more objective and robust measures of dietary exposure. In addition, nutrigenomics may have applications in the development of personalised nutrition interventions, which may facilitate larger, more appropriate and sustained changes in eating (and other lifestyle) behaviours. When combined with dried blood spot and spot urine-based measurements and with digital technologies to provide scalability, implementation of such personalised nutrition interventions could make a major contribution to better health and to reducing health inequalities worldwide.

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Conflicts of Interest

None.

Authorship

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