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NESTLÉ RESEARCH:

Harnessing the power of epigenetics for targeted nutrition

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Diet and genomes interact. Because of its continuous and lifelong impact, nutrition might be the most important environmental factor for human health. Molecular nutrition research strives to understand this interaction. Nutrigenetics addresses how an individual's genetic make-up leads to a predisposition for nutritional health; nutrigenomics (encompassing transcriptomics, proteomics and metabonomics) asks how nutrition modulates gene expression; and epigenetics provides insights into a new level of regulation involving mechanisms of development, parental gene imprinting and metabolic programming that are beyond genetic control.

Tailoring diets and nutrient compositions to the needs of consumer groups that share the same health state, life stage or life style is a main goal of current nutritional research. While there are already food products available that address the specific requirements and preferences of certain groups, these products are based on empirical research rather than on genomic or (epi)genetic assessment. Genetics and epigenetics build the scientific foundations for understanding human variability in nutritional preference, requirements, and response to diet; and furthermore, epigenetics may reveal how environmental influences can alter this variability across the life span. Inspired by the potential for personalized nutrition counseling, epigenetics research may exert a major influence on consumer diagnostics to promote health maintenance and disease prevention.

Modern nutritional research spans from the discovery of bioactive food ingredients and the investigation of their bioavailability and bioefficacy, to the assessment of individual dietary needs via genomic and genetic approaches. The present paper summarizes current findings in the field of epigenetics and gives an outlook on potential health benefits and further research needs.

Nutrition research in the 21st century

In light of the increasing importance of nutrition in the development, prevention and management of chronic diseases, modern nutrition research faces the challenge to holistically understand how dietary components interact with and within cells and organisms; and to use this knowledge to develop new strategies and products that are nutritious, safe, promote and maintain health, and prevent disease. With the advent and development of many new technologies, molecular nutrition research has opened up new doors of understanding – not only concerning which dietary components may impact an organism's health, but also how. These new insights have allowed a greater understanding of the systems involved at multiple levels, from whole animal to the cellular and molecular levels. Although challenges in this area still remain, the main focus continues to be the improvement of health through diet. Modern molecular nutritional research aims at health promotion, disease prevention, performance improvement and benefit-risk assessment[1].

Nutrition has conventionally been considered an integral part of health maintenance and disease prevention (especially in cultures such as the Chinese), and the science behind this idea was based primarily on epidemiological studies. The task now is to take this foundational research to the next scientific level – to analyze gene, protein and metabolite profiles of individuals in different health and nutritional conditions – to find early signs (biomarkers) that indicate deviations from healthy metabolism and possible targets for correcting these deviations by nutritional means[2]. A further objective is to reveal reactions towards different diets at the systemic level to demonstrate nutritional efficacy[2]. Therefore, genomics and genetics applied within the context of nutrition and health have the potential to: deliver biomarkers for health status and health effects, reveal early indicators for disease disposition, differentiate dietary responders from non-responders, and, last but not least, uncover bioactive, beneficial food components[1].

Nutrition and genes

Diet and food components are the prime environmental factors that affect the human genome, transcriptome, proteome and metabolome, and this life-long interaction largely defines the health or disease state of an individual. Most, if not all nutrients have at least indirect effects on gene and protein expression and thus, metabolism. While genomics is about expression and genetics is about the disposition of an organism, epigenetics is about development and programming.

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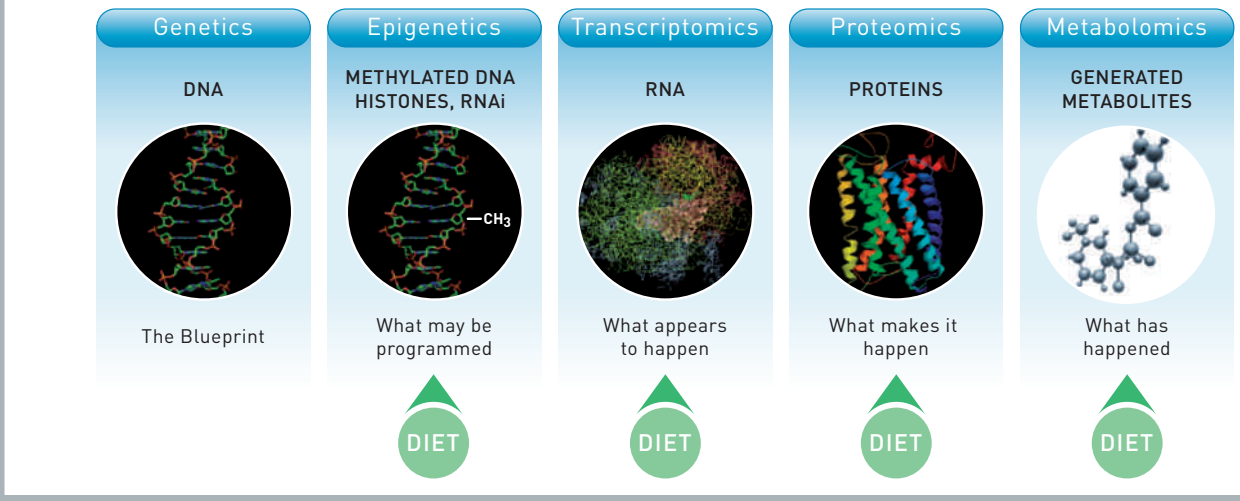

Figure 1 | Impact of diet on the genome and metabolism

Epigenetics represents an emerging set of mechanisms revealing how the environment, including food and nutrition, constantly shapes and influences the genome. With a prolonged life span and changing lifestyles in developed countries, chronic diseases have become more prevalent, and nutrigenomics, nutrigenetics and epigenetics are the key scientific platforms to advance the understanding of health maintenance and disease prevention through nutrition[3].

Current nutritional and genetic epidemiological approaches can be difficult to apply at the personalized or individual level. These methods yield risk factors derived from population studies. These risk factors are statistical estimates of the percentage of disease reduction in a population, if the risk was to be avoided or the gene variant was absent. Considering the genetic diversity of human populations, the complexity of foods, cultures and lifestyles, and the variety of metabolic processes, identifying individual risk factors poses enormous challenges for personalizing dietary advice.

Additionally, assessing the metabolic response to complex foods by Omics applications differs fundamentally from approaches in which a single active principle is typically studied (such as pharmacology or toxicology). Food contains macronutrients (carbohydrates, lipids and proteins) and micronutrients (vitamins, minerals and trace elements) that exhibit effects at RNA, protein and metabolite levels in a cell or organism. Thus, food delivers hundreds or more compounds simultaneously, causing an organ-specific response which changes across space and over time, and involves multiple cell types within an organ possibly reacting differently to the nutritional stimuli. Understanding the complexity of nutritional interactions and the mammalian system, including mRNA expression, control of the proteome, allosteric regulation, and the maintenance of metabolite pools and their interactive regulation is most challenging. Lastly, even the effects of single food components, when studied under controlled conditions in humans, are usually significantly smaller than pharmacological or toxicological effects.

Epigenetics

Whereas many earlier studies assumed that SNPs (single-nucleotide polymorphisms) were the main source of human genetic variability, an increasing body of evidence now suggests that additional layers of variability, including epigenetic regulation, such as DNA methylation, are implicated in genetic variation. Many complex diseases such as Inflammatory Bowel Disease (IBD), encompassing Crohn's Disease and Ulcerative Colitis, are associated with SNPs, particularly in chromosomal regions, but also with CNVs (copy number variants) of certain other genes[4]. Such discoveries suggest that a detailed description of the genetic background of complex diseases is a challenging but necessary objective to better prevent pathological development of such diseases.

Epigenetics literally means 'above genetics', alluding to alterations of genetic material that do not affect the DNA sequence itself: this includes DNA methylation patterns, chromatin structure and histone codes, as well as non-coding small RNAs. DNA methylation can be exerted at susceptible life phases (especially around birth) and can be sustained throughout that precise life phase, the entire lifespan, or even through the course of several generations. It seems to provide a mechanism for long-term metabolic programming of an organism in which nutrition in early life may affect health outcomes later in life[4]. Similar to DNA methylation, histones and the entire chromatin structure can affect gene expression by rendering only certain parts of the DNA material spatially accessible for transcription. Moreover, small RNAs can bind to complementary transcripts and prevent them from being processed further, thereby altering protein expression.

Two of the most comprehensively studied epigenetically regulated phenomena in mammals are X-chromosome inactivation and genomic imprinting, a genetic mechanism that controls gene expression in a parent-of-origin-specific manner[4] (i.e. gene expression depends on whether an allele is inherited from the mother or the father). Furthermore, epigenetic regulation is involved in tissue-specific gene expression and silencing.

During early embryogenesis, the mammalian genome is 'cleared' of most epigenetic marks, which are then progressively re-established during embryonic development. The epigenome is therefore most vulnerable to environmentally induced alterations during embryogenesis, when DNA synthesis is rapid and the DNA methylation pattern and chromatin structure is established for normal development. Once this has been accomplished, these epigenetic alterations are passed on to the daughter cells during somatic cell division. Epigenetic marks that are not completely erased during gametogenesis or are not well re-established during embryonic development can affect health not only in the present, but also in future generations. Additionally, epigenetic marks may be transmitted across generations, either directly through meiosis or indirectly in the next generation through replication of the conditions in which the epigenetic change occurred.

Individuals have two alleles of most genes. One is inherited from the mother and one from the father. When both copies of a gene are active, the system exhibits redundancy and is less susceptible to dysfunction. With imprinted genes, one copy is turned off epigenetically by DNA methylation. These imprinted genes are susceptible loci for disease since their normal function can be altered by a single genetic event. Furthermore, if imprinted genes are not completely turned off, epigenetic events can cause or contribute to disease.

Because epigenetic modifications alter gene expression rather than gene sequence, characterizing the expression profiles of epigenetically controlled genes should reveal epigenetic biomarkers for disease, exposure, intervention and efficacy. This could enable early diagnosis

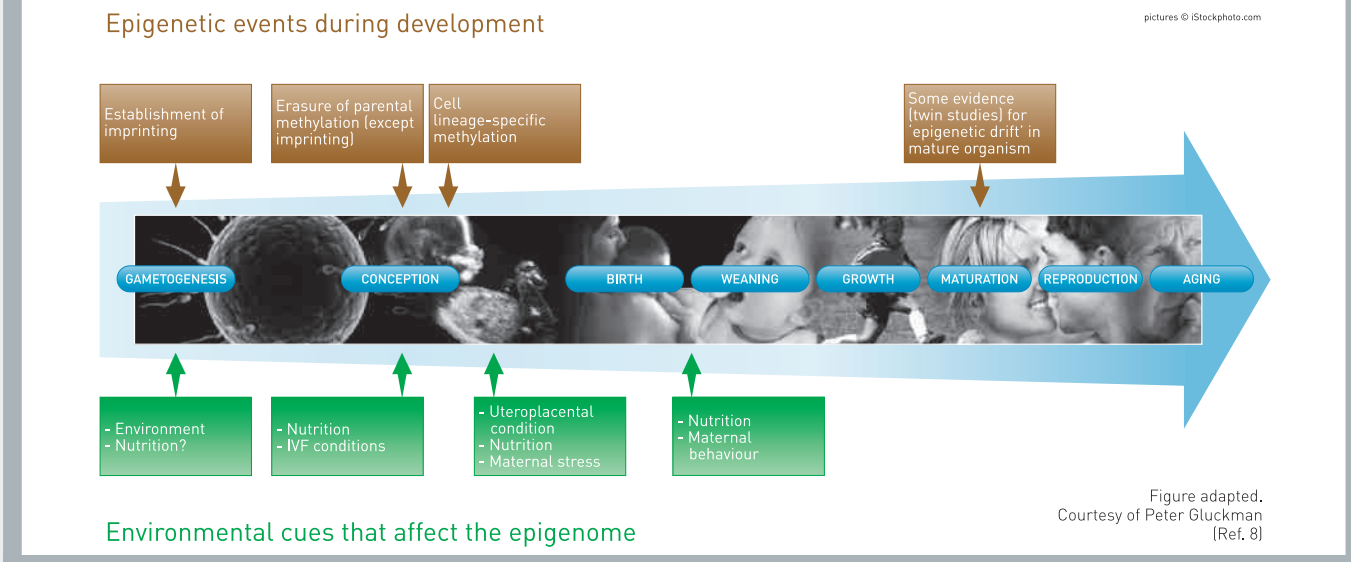

Figure 2 | Epigenetic events during development

of individuals with a propensity for adult-onset diseases and could lead to novel therapeutic approaches for the prevention and treatment of diseases, even before symptoms develop. Contemporary nutrition recommendations focus on disease prevention and health maintenance; and epigenetics may provide the means to understand and achieve these ambitious goals. Ultimately, comprehensive knowledge of the human epigenome is required, as there is great variation depending on the tissue type and stage-of-life, and also on the marked differences between individuals and species.

The relationship between epigenetics and nutrition is beginning to be revealed. One aspect is the astonishing effect that nutrition can exert on a genome. DNA methylation appears to provide a format for long-term dietary (re-)programming of the genome, suggesting that nutritional supplementation and well adapted diets at pre – and postnatal stages may exert a fundamental and long-lasting positive impact. Some evidence shows that chronic diseases and conditions in adulthood are due to persistent perturbations or influences during early-life nutrition[5].

Nutritionally induced changes may significantly affect the ontology of individual organisms. Evidence for this hypothesis has been provided by epigenetic research involving monozygotic twins[6]. Typically, such studies provide correlations between differences in phenotypic and DNA methylation patterns. While monozygous twins have the same genotype, most of these twin pairs have discordant phenotypes. One possible explanation for this is the existence of epigenetic differences[6].

Nutritional influences can alter gene expression and change phenotypes, in part by the modification of the epigenome. If these environmentally induced epigenetic modifications occur at crucial stages of life, they can potentially change behaviour, disease susceptibility and, ultimately, survival. Today's mechanistic evidence for environmental epigenetic imprinting/programming, through nutritional or other means, is mainly derived from animal models. However, human epidemiological studies increasingly point toward associations between environmental impact – especially prenatal and early postnatal – and long-term epigenetic modifications manifesting in health and disease phenotypes[7].

Early nutrition, epigenetics and late-life consequences

The foetal environment can influence susceptibility to developing chronic disorders during adulthood. Early evidence for this observation was derived from increased rates of cardiovascular disease in historical cohorts with high infant mortality rates. Further studies revealed an inverse relationship between birth weight and susceptibility to developing hypertension, cardiovascular morbidity, insulin resistance, type 2 diabetes, hyperlipidaemia and obesity[8]. It was therefore hypothesized that foetal metabolic programming under nutritionally adverse circumstances may result in increased risk of chronic disorders later in life. Other data, like those from survivors of the Dutch 'Hunger Winter' in 1944–1945, indicate that individuals exposed to unfavourable conditions *in utero* may subsequently exhibit adverse effects even if they do not have a low birth weight[7]. This observation is consistent with the complex relationship between birth weight and cardiovascular disease risk and also with the observation that metabolic dysfunction correlates more strongly with neonatal adiposity and maternal nutrition than with birth weight. Other studies focus on the role of excess nutrition during pregnancy and rapid weight gain in infants[9], the risk of which increases if foetal growth is impaired. Additionally, children exposed to hyperglycemia *in utero* and those born to obese mothers are at increased risk of developing metabolic disorders, especially type 2 diabetes[10].

Developing organisms appear to be particularly susceptible to epigenetic changes. The periconceptional period is very important, as shown by the sensitivity to suboptimal nutrition during this developmental stage[11], in which widespread reprogramming of the epigenome occurs. Nutritional constraints later in pregnancy[7], postnatal over-nutrition that leads to rapid growth[9], as well as maternal–foetal over-nutrition[12], can cause metabolic dysfunction later in life. For each of these scenarios, relevant epigenetic changes have been reported[13]. Research in animal models has demonstrated that impaired early-life nutrition and the associated epigenetic changes can be prevented[14] or reversed[13] by nutritional interventions (such as folate supplementation) or endocrinological interventions (such as neonatal leptin administration).

Primary candidates for genes that retain epigenetic memories of early life experiences are those directly associated with energy acquisition, storage and utilization. For example, the leptin gene is involved in the development of obesity and considered one of the best such gene candidates, since it encodes for a hormone which regulates energy intake and expenditure[15]. Epigenetic variation of leptin expression could possibly explain low plasma concentrations of this hormone. The promoter region of leptin is susceptible to epigenetic variation through the methylation of somatic tissues in both mice and humans[16]. It is speculated that leptin is sensitive to environmental cues and can acquire a thrifty epigenotype. Further promising candidates are genes listed in the human obesity gene map[17], imprinted genes and genes close to or disrupted by transposable elements.

Programming depends on genetics and environment

The remodeling of cells and tissues as a result of programming has been well documented, though not yet fully understood. Adipocyte hyperplasia

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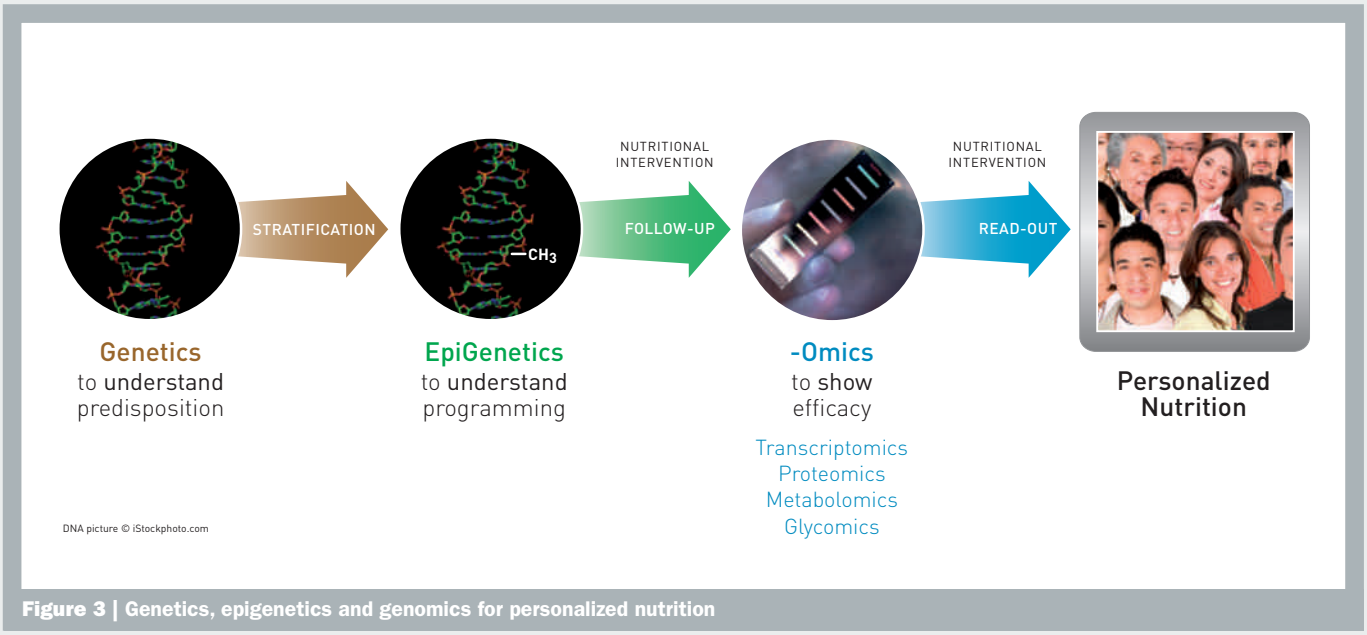

Figure 3 | Genetics, epigenetics and genomics for personalized nutrition

early in development is proposed to be one of the factors that account for the high predisposition of adult obesity in children who are overweight[18]. Animal studies have documented that specific dietary factors (e.g. ω6 and ω3 polyunsaturated fatty acids) early in life stimulate or inhibit the proliferation of adipocytes. Moreover, muscle mass is also responsive to the combination of conditioning and protein content of the diet[19]. The presence of muscle mass, its influence on whole body energy metabolism, its metabolic contribution post-training, and a larger store of amino acids in the form of muscle proteins are expected to alter an individual's reaction to environmental conditions such as diet[20].

The programming of sensory preference is perhaps the most influential but the least understood factor in the conditioning of modern humans to their habitual diets. It is arguable that in modern times, humans do not principally rely on nutrient cues to guide their food choices; instead, they rely on acquired food preferences. The remarkable sensory property of olfactory preference is the process by which liking and disliking of particular flavours are acquired as a series of contextual memories early in life[21]. This system of acquired flavour preferences underlies the cultural variation of food and cuisine around the world. This also means that flavour preferences for foods of poor nutrient quality, if acquired early in life, may guide a person's life-long habit of poor food choices, partly owing to the fact that the sensations will continue to be positively perceived[22].

Yet another mechanism by which early dietary exposure can program a person's response to diet in later life is the influence of an individual's gut microbiome (i.e. all microorganisms colonizing the human intestine). Such dietary influences can be achieved through both direct inoculation of particular microorganisms that are present in foods[23] or via the selective manipulation of subsets of microorganisms by food components that can only be fermented/utilized by certain bacterial populations[24]. Stimulated by the astonishing discoveries of Gordon *et al.* on the influence of specific bacteria on energy metabolism and obesity predisposition[25], the gut microbiota is increasingly viewed as a pivotal factor in human metabolism, immunity, sensation, disease resistance, inflammation, and comfort.

Nestlé's research in nutritional epigenetics and genomics

Nestlé Research is keenly interested in nutritional epigenetics and genomics because we want to better understand and leverage the interplay beween diet, nutrients and the human genome for short-term efficacy and long-term benefits. At the Nestlé Research Center, we utilize a variety of research methods to build knowledge on this complex topic. For example, –Omics (transcriptomics, proteomics, metabolomics) methods can show dietary efficacy and nutritional mechanisms; genetics can clarify individual predisposition and stratify cohorts in clinical studies; and epigenetics may help us better understand metabolic programming.

G proteins

Regarding genetics research, we target hypothesis-driven approaches to assess variants of specific, metabolically relevant genes in subjects enrolled in nutritional interventions. G proteins, for example, are expressed in all cells of the human body, and their main role is to translate signals from the cell surface into a cellular response. The heterotrimeric composition of G proteins, consisting of α-, β-, and γ –subunits, determines their receptor and effector specificity. Due to the pivotal role of G proteins in virtually all intracellular signal transduction processes, metabolically relevant SNPs in G proteins have been characterized. Some of these gene variants may be implicated in weight regulation. For these reasons, we genotype healthy adult volunteers enrolled in weight management trials at the G protein level.

Long-chain polyunsaturated fatty acids

Another case of genetically influenced dietary response is represented by the health effects of ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs). It is suggested that individual response to LC-PUFAs depends on the host's PPAR (peroxisome proliferator-activated receptor) α/γ-genotype. ω-3 LC-PUFAs are associated with benefits for lipid metabolism, insulin sensitivity and inflammation, and these PUFAs bind directly to the PPAR transcription factors. Human studies have shown that the effect of this PPAR-PUFA interaction is modulated by polymorphisms in the *PPAR* genes. Therefore, we leverage this interdependence to better understand the genotype-dependent health benefits of functional lipids.

Weight management for humans

Caloric restriction is to date the only nutritional intervention that has been shown to have a measurable effect on life span across many species, including mammals. In an *in vivo* study, we investigated the effects of caloric restriction and specific nutrients at the whole transcriptome level in different tissues. We are currently addressing the epigenetic dimension of this study through pursuit of this question: `which long-term gene expression changes are exhibited by DNA methylation changes?' Then we can focus on the nutrients that appear to exhibit transcriptome profiles similar to that of caloric restriction.

Weight management for pets

Maintaining a lean body mass phenotype is important to overall health, for humans as well as companion animals. The maintenance of lean body mass plays a critical role in weight management. With increasing obesity prevalence, along with its associated health risks, the maintenance of healthy weight must be fully understood and

applied. We have recently identified common molecular mechanisms associated with lean body mass phenotypes in order to understand and promote these changes via nutritional solutions. These phenotypes were induced using three different treatments which corresponded to changes associated with lean body mass, rather than the individual treatment alone. In addition to biochemical and gene expression changes, epigenetic changes are now being identified.

Taste sensomics

Dietary behaviours and nutritional phenotypes, especially their representation and frequencies within a given population, are of great interest to Nestlé Research. As previously mentioned, the predisposition to certain nutritionally linked phenotypes such as overweight, diabetes or osteoporosis, can be partially anticipated through the genetic blueprint.

However, what about behavioural traits such as the detection thresholds for taste or preferences for specific foods? With the tremendous insight achieved over the past decade on the molecular machinery of chemosensory perception — tasting and smelling — the pursuit of related genetic variants is straightforward.

At the Nestlé Research Center, a genome wide association study (GWAS) has been initiated to study a panel of several hundred participants of multi-ethnic backgrounds. The study aims to gather data about participants' sensory characteristics, specifically their taste and metabolic phenotypes.

Preliminary study results have already revealed interesting findings. The well known association between detection threshold for the bitter tasting propylthiouracil (PROP) or phenylthiocarbamide (PTC) and the bitter taste receptor T2R38 has been easily detected in a small cohort of 100 subjects. In addition, it was discovered that a set of SNPs indicative for sensitivity to quinine are associated with a group of specific bitter taste receptors (T2Rs). Further associations are emerging from this data set. Nestlé's ambition is to use the final results to build a specific subject panel, characterized at the molecular level, to conduct consumer testing and targeted clinical intervention studies. Moreover, the results obtained through this GWAS will build a foundation for understanding more complex mechanisms such as the human taste perception of salt and its link to dietary salt intake and hypertension. With this knowledge, tangible solutions for long-term sodium reduction are feasible.

Finally, the association between a given set of chemosensory phenotypes with specific genotypes, and possibly even a linked set of metabolic phenotypes, will enable the definition of population clusters with certain sensory characteristics and food perceptions. In the future, we would like to perform an analogous study in cats or dogs to gain unprecedented insight into the inherited traits of pets, likely including the dietary preferences and nutritional needs for different breeds.

Our perspective

The genomic sciences have delivered proof that what is considered an 'optimal diet' varies considerably between humans. As nutrigenomics and nutri(epi)genetics build the scientific foundation for this concept, in addition to sequencing and methylation technologies becoming more widespread and readily accessible, people may gain valuable information from their personal (epi)genetic code.

Furthermore, as the ability of scientific tools to distinguish human physiological differences meets the industrial capability to deliver individual solutions, food and nutrition will become increasingly personalized. This process is not a revolution, but rather reflects the continued diversification of food that has been ongoing for centuries. Consumers will benefit from practical applications such as food personalization based on validated nutritional solutions for specific subsets of the population. Infants, pregnant and lactating women, active or sedentary adults, athletes, frail elderly, and people who suffer from inherited or acquired diseases – respectively represent large consumer groups with specialized food requirements and personal preferences such as taste, texture, and appearance. Developing nutritional interventions to meet the specific needs of these various consumer groups should parallel similar approaches in personalized medicine.

Humans are not only diverse with respect to (epi)genetics; there are countless variations on biological and physiological levels. A synergistic approach combining phenotyping, genotyping, epigenetic characterisation and holistic investigations of the metabolism is necessary to fully understand the complex interactions between genes, nutrition and environment. Nestlé is committed to the pursuit of this scientific knowledge to gain deeper insights and to leverage the benefits of diet and nutrition for health, wellness, and improved quality of life for people of all ages and stages of life.

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