CHAPTER 7

ABSTRACT

That epigenetic changes during lifetime (i.e. those with a biological purpose, epigenetic drift and epigenetic clocks) depend on a complex mixture of factors is well-known, as is the fact that, depending on the loci, they can be modulated by genetic and/or external factors, lifestyle being one of the most important. However, the underlying molecular mechanism remains largely unknown and addressing this will be an important challenge for CSIC in this field.

KEYWORDS

life style | aging | epigenetic clocks | physical exercise | nutrigenomics | transgenerational inheritance

CHAPTER 7

EPIGENOMICS AND LIFE STYLE

Coordinators
Mario Fernández Fraga
(CINN, Oviedo, Coordinator)
Sonia Ramos Rivero
(ICTAN, Madrid, Deputy Coordinator)

Participating researchers and research centers (in alphabetical order)
Laura Bravo Clemente
(ICTAN, Madrid)
Carmen Collado
(IATA, Valencia)
Raúl Fernández Pérez
(CINN, Oviedo)
Belén Gomara
(IQOG, Madrid)
Rocío González Urdingio
(CINN, Oviedo)
María Ángeles Martín Arribas
(ICTAN, Madrid)

EXECUTIVE SUMMARY

The past two decades have served to consolidate the concept that epigenetic modifications change during lifetime. Certainly, numerous scientific works have described several epigenetic signatures associated with increased age and, remarkably, epigenetic "clocks" have been recently developed which can predict biological age by measuring levels of epigenetic marks such as DNA methylation. These clocks also serve as biomarkers of physiology because they are altered in pathological states. However, although these predictors of chronological age have been well characterized, the underlying molecular mechanisms are still largely unknown. There is thus an urgent need to describe these molecular mechanisms and to determine whether epigenetic clocks are a cause or consequence of the increase of age.

In recent years, numerous (mainly descriptive) works have proposed that epigenetic changes during lifetime can be modulated, at least in part, by external stimuli. It has also been proposed that lifestyle during pregnancy can program the epigenome during embryonic development and determine certain disease phenotypes in adult life. If true, this would imply that lifestyle might shape health and disease phenotypes through epigenetic mechanisms. Moreover, at present it is unclear whether transgenerational epigenetic inheritance or intrauterine exposures influence offspring's health and disease susceptibility. Therefore, the role of epigenetics in transgenerational inheritance requires to be explored.

Diet is one of the most important factors in lifestyle and has the potential to modulate gene expression programs through epigenetic mechanism, thus affecting individual health and life expectancy. A challenge for the future will be to determine both how nutrients and bioactive components in food influence epigenetic function and the underlying molecular mechanisms involved. This will be important in order to describe new effective nutrition-based preventative and therapeutic approaches. In this way, nutritional epigenetics can be instrumental in developing personalized programs that contribute to reducing the risk of disease and improving health. Moreover, in addition to beneficial nutrients, foods might also contain variable amounts of pollutants which exhibit different mechanisms of toxicity and bioactivity. Thus, nutrigenomic studies in the future should consider not only the beneficial effects of nutrients but also the possible harmful effects of contaminants present in foodstuffs.

Another central component of lifestyle is physical activity. Indeed, it is well known that regular exercise influence health and prevent disorders, such as cardiovascular and metabolic diseases or cancer. As with diet, epigenetic mechanisms have also been proposed to be the molecular link between these health benefits and physical activity. However, as most of the studies are focused on specific candidate genes in target tissues, it is necessary to carry out extensive genome-wide analysis of epigenetic modifications in the future. It will be also necessary to identify specific epigenomic signatures associated with different types of physical activity (e.g., gentle aerobics, strength or endurance activity, etc.).

In addition to diet and physical activity, other important aspects of lifestyle, such as alcohol and recreational drugs use, pharmacological treatments, etc., must also be taken into consideration. For all these factors, future research should not only be limited to describing epigenetic changes in response to a given environmental cue, but also to trying to identify the functional and physiological consequences of these changes.

Finally, because epigenetic changes are in principle reversible, they provide an avenue for the development of therapies to counter complex processes, such as aging. Among the challenges for the future are the identification of the alterations involved, the separation of the biologically relevant changes from the rest of the environmentally-induced noise, the design of the biological tools to reprogram epigenetic alterations and, importantly, the development of instruments, such as epigenetic clocks, which will allow us to ascertain whether our interventions have an effect on our life expectancy with a healthy aging.

7.1–INTRODUCTION AND GENERAL DESCRIPTION

7.1.1 Epigenetic changes during lifetime (epigenetic drift vs epigenetic clocks)

Aging is a universal phenomenon in which biological functions gradually decline, ultimately leading to death. At the cellular level, these changes influence a wide array of molecular pathways and include both genetic and epigenetic alterations (López-Otín et al., 2013). Indeed, epigenetic mechanisms may, at least in part, mediate aging features such as genome instability or transcriptional noise (Pal and Tyler, 2016). These alterations have been described at all levels of epigenetic regulation, with DNA methylation and histone modification being those most widely studied, both in human and model organisms (Pal and Tyler, 2016). DNA methylation, in particular, has been investigated in humans, and this has led to some accepted notions regarding the gradual changes observed during lifetime: a global loss of DNA methylation at intergenic and repetitive regions, local gains at CpG-dense regions and, in general, an increase in intra- and inter-individual variability in DNA methylation patterns (Huidobro et al., 2013; Jones et al., 2015). This last observation, commonly referred to as "epigenetic drift", underscores the idea that the epigenetic changes observed during the aging process are comprised of a combination of functional and stochastic alterations, which in addition may or may not be significant in the regulation of gene expression (Tejedor and Fraga, 2017). Because epigenetic alterations can have innumerable origins, including environmental elements such as lifestyle factors, it is crucial to be able to separate specific and non-specific variations (Feil and Fraga, 2012).

Within this scenario, owing to the development of genome-scale microarray and NGS (next generation sequencing) technologies, the concept of the "epigenetic clock" has recently emerged in the field. Epigenetic clocks consist in mathematical models which use DNA methylation information to predict chronological age with an unprecedented level of precision (Horvath and Raj, 2018). Moreover, the consistent behavior across lifespan of the CpG sites involved sets them apart from those implicated in age-related epigenetic drift (Jones et al., 2015). Both aging-associated epigenetic changes and the recently characterized epigenetic clocks may help explain the molecular mechanisms involved in defining the phenotypic variability observed between individuals at the physiological as well as the pathological level. In order to do so, however, they need to be effectively disentangled from stochastic epigenetic variation.

7.1.2 Nutrigenomics and nutriepigenomics

Nutrition is considered one of the most impacting life style factors able to affect the genome and epigenome. Nutrigenomic describes the interaction between nutrition and genes to understand how specific food constituents or dietary regimes may affect human health. The novel discipline of nutriepigenetics integrate the knowlege of nutrigenomic and the effect of diet or dietary compounds in gene expression programs through epigenetic mechanisms. Diet, foods and its components affect the genome and epigenome and have the potential to modulate critical metabolic pathways influencing individual health and life expectancy. A link between certain dietary patterns and diverse noncommunicable diseases (cancer, cardiovascular disease, obesity, diabetes) has been suggested. These associations constitute a starting point about how diets and ultimately foods might modify the genome and epigenome and, consequently, the proteome and metabolome. Therefore, each dietary pattern might provide diferent epigenetic and genetic signatures, which could be associated to a healthy status or disease susceptibility. Nevertheless, nutritional epigenomics is a quite recent research area and present data on the precise effects of diets, foods or food components on the epigenome are very limited. At present, most of these studies are descriptive and the epigenetic modifications as well as the underlying molecular mechanisms remain still largely unknown. In addition, most of nutritional epigenetic works have focused on DNA methylation, while post translational histone modifications and miRNA expression are even less analyzed.

7.1.3 Pollutants present in foodstuffs

Air and dust inhalation and food ingestion are considered the main routes of exposure to toxic chemical compounds for the general population. In the case of lipophilic compounds, the ingestion of food is the most important source of exposure. Many toxic environmental pollutants with endocrine-disrupting properties are lipophilic. Therefore, they bioconcentrate in living organisms and bioaccumulate through food webs making humans the organisms receiving the highest impacts. In addition, agrochemicals used for pest control, veterinary drugs employed on farming, migrating compounds related to food contact materials (FCMs), food additives (ranging from food colorings, preservatives, and stabilizing agents, to bioactive compounds), and contaminants introduced or formed during food storage (such as mycotoxins and biogenic amines) and processing (like acrylamide) reach humans through the diet. Many of these compounds have been demonstrated to be toxic and exert epigenetic modulation.

7.1.4 Precision nutrition in human health and disease prevention

Nutrition plays a central role in the prevention of many chronic pathologies, with diet being a key modifiable factor that may influence the incidence of highly prevalent metabolic disorders, both monogenic (e.g. celiac disease, lactose intolerance) and polygenic (e.g. type 2 diabetes, obesity, metabolic syndrome, cardiovascular diseases), or certain types of cancer. To complicate things further, some diseases may be associated to both monogenic and polygenic risk factors. For instance, obesity, which incidence is steadily increasing and is associated to many comorbidities (diabetes, dyslipemia, hypertension, inflammation, etc.), may represent a symptom of up to 40 monogenic diseases and chromosomal abnormalities, but obesity may also depend on numerous genetic variants, with more than 600 genes and DNA regions associated to human obesity by GWAS. This shows the complexity of studying multifaceted polygenic traits related to numerous physiological pathways.

Besides, the potential therapeutic role of nutrition in the prevention of chronic degenerative diseases is multiple and complex. Not only nutrients in foods, but especially bioactive food components (polyphenols, carotenoids, phytosterols, isothiocyanates, glucosinolates, ω-3/ω-6, etc.) may have a marked effect on health. Many of these bioactive compounds will have actions at various molecular levels, from DNA expression, pretranscriptional modifications, affecting protein functionality, metabolic processes, etc. These nutri(epi) genomic implications of food components are also accompanied by nutri(epi)genetic factors affecting crucial steps as eating preferences, ADME (absorption, distribution, metabolism and excretion), metabolic pathways and, in the end, the individual's phenotype and clinical response. In addition, the gut microbiota may be affected by these food components and modify their activity through catabolic modifications of the ingested molecules.

All this results in large variability in the individual response to diets and foods, and the need for an integrative molecular and -omic approach to nutrition. Like precision medicine, precision nutrition also requires an individual approach to the person based on the 4P principles (personalized, predictive, preventative, participative) for which genetics becomes essential.

7.1.5 The impact of diet-microbiota and interactions in precision nutrition

Nutrition plays a relevant role in human health. Molecular nutritional research has been defined as "the science that studies the effects of nutrients, food and its components, on the whole physiology and the state of good health at the molecular and cellular level". In the future, nutrition research should progress beyond the one-size-fits-all diet towards the study of the personalized host response to diet. This concept needs to integrate both biological (microbiome, epigenome, metabolome, genome, etc.) and environmental variables (diet, physical activity, drug intake, xenobiotics, infections, stress, etc.) to obtain detailed predictions of the individual's responses to specific nutrients and other dietary bioactive compounds.

7.1.6 Physical activity

The beneficial effect of physical activity on health is well known (Fiuza-Luces et al., 2013; Neufer et al., 2015). A physically active lifestyle and regular exercise help to regulate blood pressure and metabolism, modulate homeostasis, and generally contribute to improving health and preventing diseases(Booth et al., 2012). Lack of physical exercise influences health throughout life, and is associated with higher premature mortality, coronary heart disease, type 2 diabetes, colon and breast cancer, as well as obesity (Booth et al., 2012; Nieman et al., 2019; Fernandez-Sanles et al., 2020). The benefits of exercise on memory and cognition are also known (Hillman et al., 2008; Fernandes et al., 2017), which highlights its importance in the maintenance of physical and mental health during life.

Although the biological mechanisms that regulate the beneficial effects of exercise on health are not fully understood, an important role for epigenetic marks has been proposed, because epigenetics can represent the molecular link explaining how the environment affects our genes (Feil and Fraga, 2012). Epigenetic changes associated with physical activity have been studied at different levels in terms of the tissue type and type of epigenetic mark analyzed, although they have mainly been studied in blood cells, in muscle and adipose tissue, and in brain tissue in murine models (Elsner et al., 2011; Seaborne et al., 2018; Nielsen et al., 2010). Within this context, DNA methylation is the most studied epigenetic mark, both at the global and the gene-specific level (Fernandez-Salnes et al., 2020; Seaborne et al., 2018a; Ronn et al., 2013; Schenk et al., 2019), followed by miRNAs and posttranslational histone marks (Elsner et al., 2011; Nielsen et al., 2010; Pandorf et al., 2009; Melo et al., 2015).

The emergence of -omic technologies in general, and epigenomics in particular, open up new avenues to study in more detail the effects of exercise on the epigenome, and especially the possible functional effects that these epigenetic changes may have on health.

7.1.7 Alcohol and drugs of abuse

In the same manner as our diet, during our lives we find ourselves exposed to many other types of compounds. The National Institute of Statistics in Spain estimated in 2017 that more than 20% of people aged 15 or older were daily smokers, and a similar proportion of citizens drank alcohol on a weekly basis (Instituto Nacional de Estadística, 2017). Alcohol and tobacco have well-known and wide-ranging consequences on our health, and thus it should come as no surprise that their consumption has been linked to epigenetic changes. Because of their legal status and prevalence in the population, most of the research has been carried out on these two substances. Regarding tobacco, it has to be taken into account that the thousands of different hazardous compounds present in its smoke can affect a myriad of pathways (Talhout et al., 2011), while alcohol effects pertain to those of ethanol metabolism.

7.2—IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

7.2.1 Epigenetic changes during lifetime (epigenetic drift vs epigenetic clocks)

The characterization of epigenetic changes with aging in the context of epigenetic drift is only starting to be addressed. A significant decrease in the cost of microarray technologies has allowed the development of large-scale studies, which can better capture more subtle epigenetic changes within the scenario of noisy inter-individual variability. In this same vein, large-cohort studies have revealed that variability in DNA methylation changes are linked to aging associated molecular pathways (BIOS consortium et al., 2016), thus providing an explanation of the processes involved in aging, and have also served to identify particular genetic loci which may provide key avenues for anti-aging intervention (McCartney et al., 2020).

In addition, epigenetic clocks also show great potential as tools in the investigation of aging and aging-related disease. Epigenetic age has been shown to be accelerated in association with a wide range of pathologies, a significant observation which serves to show that the clocks can be used as biomarkers

of disease (Horvath and Raj, 2018). The fact that same-age individuals manifest different epigenetic ages points towards the notion that these models are capturing, at least in part, "biological aging", which can be thought of as the general physiological status in relation to mortality risk and comorbidities (Bell et al., 2019). As such, a new generation of clocks focused on biological age is already being developed to better capture disease associations (Lu et al., 2019; Levine et al., 2018). Perhaps the most promising of their applications is the fact that they could be used to evaluate the success or failure of antiaging interventions.

7.2.2 Nutrigenomics and nutriepigenomics

Future work in the field of nutrition and epigenetics has the potential to provide significant benefit to public health. Deciphering the epigenetic signatures triggered by bioactive food components might lead to personalized nutritional interventions that takes into account genetic/epigenetic information. Nutritional epigenetics represent a safe potential and innovative strategy for the prevention or treatment of many prevalent chronic diseases that are close related to epigenetic modifications.

7.2.3 Pollutants present in foodstuffs

The presence of pollutants in foodstuffs is routinely controlled by the sanitary authorities, to ensure food safety. However, research on the effects of these substances at the epigenetic and genomic level is still rather limited, although mandatory for proper food legislation and regulation. An additional difficulty associated to the presence of industrial and agricultural pollutants and veterinarian drugs is that, when the toxicity of these chemicals is established, they are rapidly substituted by alternative products, making previous routine controls and toxicity data render obsolete and forcing the scientific community and food authorities to develop new analytical methodologies and strategies for their identification, determination, risk evaluation, and routine monitoring.

Therefore, the future of nutrigenomic goes through considering not only the beneficial effects of nutrients but also the possible harmful effects derived from either natural or anthropogenic contaminants present in foodstuffs. This is a complex challenge since many known contaminants have been associated with a high incidence and prevalence of different endocrine-related disorders in humans, but also because of the constant introduction in the food web of new substances that can also impact the (epi)genome.

7.2.4 Precision nutrition in human health and disease prevention

There is an increasing interest in precision nutrition for its potential in the prevention and treatment of chronic non-communicable diseases, both monogenic and polygenic. GWAS and other genetic studies have identified over 15000 SNPs associated with numerous pathologies and traits. Besides genetic polymorphisms, epigenetic modifications, which are tissue-specific, highly affected by environmental/lifestyle factors (including the diet), and reversible but also transgenerationally inheritable, complicates the differential susceptibility and responsiveness of individuals to diet-related pathologies and dietary interventions. Along with these factors, ncRNA also play an important role, since lncRNA and specially miRNA shed in extracellular vesicles can have paracrine or endocrine-like effects in different target tissues and organs. In line with this, the role of ncRNA ingested with foods should also be considered as potentially important external epigenetic modifiers, since in general the impact of ncRNA on metabolic pathways and regulatory networks is still little known.

Bearing this in mind, the identification of genetic variants or epigenetic marks that predispose individuals to suffer from certain metabolic-related diseases is key for precision medicine and precision nutrition alike to estimate disease risk and design preventive strategies. Similarly, recognizing relevant gene-diet interactions will allow to identify responsive and nonresponsive individuals for specific dietetic interventions, which in turn would permit designing personalized recommendations to maximize the benefit of nutritional interventions. This genotype-directed nutrition will be useful not only for individual personalized dietary advices, but will also improve public health recommendations and the design of nutrition solutions, including functional foods and nutraceuticals.

7.2.5 The impact of diet-microbiota and interactions in precision nutrition

Our diet and lifestyle, as well as other habits (e.g. sleep patterns) and exposures (e.g. stress, pollutants, blue light, food chemicals and plastics), have changed dramatically over the last few decades. Exposure to unhealthy food, inadequate dietary patterns, such as excess or deficiency of macro- and micronutrients, may increase the risk of non-communicable diseases (NCDs). This exposure may act through several potential mechanisms, including the modulation of the microbiome, but also affecting the metabolome and epigenetic regulation, cellular and physiological routes and the immune system,

which affect host response and health, thereby increasing the risk of NCDs. Most of these mechanisms remain unclear, and thus there is an urgent need to generate scientific evidence through human studies.

A strong link has been observed between microbiota dysbiosis and the risk of NCDs, such as allergies, obesity, diabetes, immune-related problems and cardiovascular diseases, which have resulted in an increasing global burden that requires urgent action. According to the World Health Organization (WHO), NCDs are the cause of > 41 million deaths every year (accounting for 71% of all deaths worldwide) and thereby have a dramatic impact at the societal and economic levels.

Interestingly, the period comprising gestation and the first years of life is considered to be the most critical period in terms of the risk of developing NCDs. The impact of perinatal nutrition on infants' microbiome development has become the subject of significant interest but there is scarce information concerning women's microbiome and nutrition prenatally and during gestation. Maternal environmental exposures, including diet and microbes, can promote long-lasting or even induce permanent changes in foetal physiology, thereby exerting an impact on the risk of disease in later life. Nutrition also exerts both short- and long-term effects on human health through programming immunological, metabolic and microbiological development. Furthermore, the interaction between nutrients-microbiota-host towards epigenetic regulation would impact foetal development and also, infant and maternal health outcomes.

Maternal microbiota represents the main microbial source for infants and specific microbial transference from mothers to their offspring occurs at birth and during lactation.

Maternal microbial dysbiosis during pregnancy is transferred to the neonate, resulting in the inadequate development of the microbial inoculum as well as immune and metabolic effects with future unfavorable health outcomes. Currently, it is unclear whether transgenerational epigenetic inheritance or intrauterine exposures influence the offspring's health and disease susceptibility. Diet plays a major role in shaping the gut microbiota, while nutrient-microbiota interactions influence the host's health outcomes, thereby having critical implications for health; indeed, "We are what we eat". Microbiota is involved in dietary digestion, nutrient absorption, immune system training, pathogen and toxin protection as well as production of specific

compounds (SCFAs, vitamins, hormones, neurotransmitters, etc.). Microbiota interact with the metabolism of dietary carbohydrates, proteins, plant polyphenols, bile acids and vitamins. Additional studies are needed to identify which foods, macro- and micro-nutrients and specific dietary compounds influence the microbiota. Specific dietary nutrients, such as fiber, methyl donors (betaine, methionine, and choline), folate and other group B vitamins (B2, B6 and B12), are linked to microbiota. Humans need these nutrients, and besides dietary sources, there is evidence that the gut microbiota are also a source of essential nutrients for the host (e.g., folate and other B-vitamins). Remarkably, most of these nutrients play a crucial role in epigenetic regulation and can have an impact on the human epigenome.

7.2.6 Physical activity

The application of new high-throughput technologies in the -omics era will not only allow the identification of exercise-induced molecular changes in the epigenome, but will also help to facilitate our understanding of the mechanisms that contribute to improving health. The value of analyzing in detail the changes or epigenetic alterations induced by exercise are unquestionable. It will help us to understand the beneficial effects of exercise on disease prevention and treatment and will offer new potential therapeutic targets. It will provide clues to how to combat cognitive diseases associated with aging such as Alzheimer's, given that the results obtained so far indicate the beneficial effect of exercise on memory and cognition (Hillman et al., 2008; Fernandes et al., 2017). A more in-depth knowledge of these alterations will also generate information on molecular markers, indicators of optimal sports performance that are of great interest to professional athletes. And last but not least, it will help us clarify how exercise-induced epigenetic changes are sustained over time (epigenetic memory) (Sharples et al., 2016; Seaborne et al., 2018b), and even whether these epigenetic changes are inherited and thus have beneficial effects on the offspring (Segabizani et al., 2019; Spinder et al., 2019).

7.2.7 Alcohol and drugs of abuse

Currently, there is substantial evidence that tobacco smoking leads to DNA methylation changes in human (Lee and Pausova, 2013). Microarray association studies have led to the identification of recurrent epigenetic markers such as the F2RL3 gene, related to vascular functions, or the xenobiotic metabolism AhR gene (Breitling et al., 2011; Shenker et al., 2013; Sun et al., 2013). These and other observations suggest that the epigenetic alterations involved could be important in the molecular mechanisms associated to the tobacco

adverse effects. On the other hand, the epigenetic effects of alcohol have been studied in a more mechanistic manner, especially because ethanol is known to disrupt one-carbon metabolism, and thus may influence methylgroup usage by DNA methyltransferases (Pérez et al., 2019; Ron and Messing, 2011). As in the case of tobacco, the most recent studies are starting to apply genome-wide technologies to screen for alcohol-associated genetic loci (Zhang and Gelernter, 2017). On the whole, genomescale association studies have the potential to identify biomarkers of compound usage and potential pathways involved in the etiology of drug-related pathology, while mechanistic studies are particularly relevant to the latter application.

7.3–KEY CHALLENGING POINTS

7.3.1 Understanding of the epigenetic clock

What is the biological significance of the existence of CpG sites whose methylation status reflects chronological or biological aging? The loci which make up the clocks have been associated with specific genomic features, such as polycomb-associated sites (Raj and Horvath, 2020) but there are still no clear-cut links to any specific biological process. It remains to be clarified whether epigenetic clocks are "drivers" of aging or mere "passengers" reflecting the footprints of other processes, an observation which will be relevant to the design of anti-aging interventions.

7.3.2 Measuring and identifying variability

The characterization of epigenetic variability is key to the identification and separation of epigenetic drift from other functional or disease-associated changes. Large-scale studies facilitated by the integration of public datasets will throw light on this scenario. However, different interpretations and measurements of variability coexist in the field (BIOS consortium et al., 2016; Gentilini et al., 2015) and their biological significance will need to be better defined.

7.3.3 Delineating chronological and biological aging

Medical advances leading to increases in lifespan will make no sense without corresponding increases in healthspan. Both a more global and a more specific characterization of biological aging and its biomarkers is needed in order to be able to confront this challenge, and more refined epigenetic clocks will surely be crucial in this scenario (Bell et al., 2019; Partridge et al., 2018).

7.3.4 Investigating epigenetic transgenerational inheritance in mammals

It has been proposed that the epigenome susceptibility to adapt to lifestyle factors, including nutrition, is different through the lifespan of an organism, being more sensitive to changes at early stages (pre- and neonatal period) (Kanherkar et al., 2014). Also, epigenomic modifications seem to be reversible, although it has been postulated that these alterations can passed through generations. In this regard, experimental data in humans have demonstrated that metabolic disorders (undernutrition and maternal obesity) during early development periods (pregnancy) generate an abnormal developmental ambient which could modify the epigenome and predispose the offspring to metabolic diseases later in life (Tobi et al., 2014; de Rooij et al., 2006a,b). This has been explained by the so-called "Developmental Origins of Health and Disease" hypothesis in which a transgenerational epigenetic inheritance is proposed (Gluckman & Hanson, 2004) This hypothesis is also focussed on the effects of pre- and peri-conceptual nutrition in both parents which would point out to the possibility of providing a favorable nutritional status of development to obtain beneficial epigenetic changes (Fleming et al., 2018). However, at present it remains equivocal whether transgenerational epigenetic inheritance or intrauterine exposures influence offspring's health and disease susceptibility. These challenges deserve future investigations, and will require observation and tracking multiple generations.

7.3.5 Dissecting the role of diet-induced epigenetic modifications in human health and disease

Nutrition can also affect health and predispose to disease susceptibility later in life. Evidences from dietary intervention studies, as well as from researches in which the effect of food components have been assayed in humans and animal experimental models, have suggested that dietary components (nutrients and bioactive compounds) exert different biological activities that could render protective effects against different non-communicable diseases and lead to a healthier ageing. However, it is challenging to elucidate their molecular mechanisms and associated epigenetic modifications. Interestingly, this approach to evaluate the potential benefits of a nutrient or food component could be useful to identify epigenetic changes and define early biomarkers of disease. This will also be important to desing new effective nutritionbased preventative and therapeutic approaches, which will contribute to improve health and to reduce the risk of disease. Yet nutrigenomic studies are complex and proving a causation from an association is complicated. It is challenging to

identify which components of a disease phenotype are related to nutrition, except for those diseases caused by a single gene defect. In addition, it is also complicated to understand how humans respond to specific diets or nutrients and to detemine the component/s in food responsible/s for an action. Indeed, there are many components in the diet and these substances interact causing multiple metabolic changes, which may even differ depending on the way of intake for the same food (Nicodemus-Johnson & Sinnott, 2017). In addition, it should be taken into account that the genome and the epigenome might interact, i.e. understand how epigenomic modifications could alter gene expression, and regulate the impact of nutrition constitutes also a great challenge that will enable the fisrt steps towards the precision nutrition in disease prevention.

7.3.6 Understanding the impact of exogenous miRNA

miRNA have been identified in biological fluids (blood, human breast milk and milk from other species). However, the influence of these exogenous miRNAs has not been studied in depth despite these epigenetic elements are detected in most foodstuffs and are frequently conserved across species (Xia et al., 2011.; Ledda et al., 2020; Mal et al., 2018) Understanding the potential impact of exogenous miRNA on human epigenetics and their possible influence on health and disease susceptibility is decisive. Indeed, a proved benefitial or detrimental effect might lead to changes in food manufacturing, processing and/or cooking. In addition, because of their generalized presence in foods, miRNA have been suggested as potential biomarkers and/or communication elements (Benmoussa & Provost 2019). All this might enable a new therapy approach against non-communicable diseases and/or promotion of a healthier ageing.

7.3.8 Uncovering the genetic and epigenetic consequences of food chemicals

It is well known that thousands of toxic substances can be present in foodstuffs as a consequence of the production, transport, processing, packaging, and storage practices, but also due to the natural impact of the residual environmental contamination. The number of chemical substances that has been found to have epigenetic toxicity is continuously rising (Marczylo et al., 2016). On the other hand, our knowledge of the epigenetic and genetic effects of old and new chemicals is expected to increase significantly thanks to recent advances on the analytical techniques and methodologies. Besides, different diseases have shown to be transgenerationally transmitted in animal models (Guerrero-Bosagna & Jensen, 2015). All this new knowledge pushes research toward studies focused on both the epigenetic changes observed nowadays

and the transgenerational consequences of current human exposure to toxic chemicals related to the ingestion of food. To reach this objective it is essential to develop and transfer appropriate determination methodologies based on state-of-the-art instrumentation from the research to the official routine laboratories for the accurate, fast, and green determination of well-known regulated chemicals in food, but also of emerging and new toxics compounds that could be introduced in any of the steps of the food chain. To achieve this latter goal, non-target approaches must be implemented in routine controls, which makes mandatory the adaptation of the analytical methodologies in use to fulfill current demands regarding selectivity and sensitivity. Such approaches will allow achieving the comprehensive experimental data on food exposure that will allow proper correlation with epigenetic and genetic changes observed in the population.

7.3.8 Moving towards precision and personalized nutrition

The final objective of precision nutrition is being able to provide personalized dietary advice taking into account individual responses to maintain health and prevent disease. To this, advance in the following points is required:

- Large population studies based on well and thoroughly characterized populations (clinical anamnesis, sex, age, lifestyle, (epi)genetics, microbiota) are needed to advance in the identification of determinants of individual variability in the response to specific dietary interventions for the different diet-related traits. Clinical trials and cohort studies.
- Better knowledge of genetic determinants of ADME (for nutrients and bioactive food components) and food preferences, as well as dietary requirements influenced by SNPs. Comprehensive list of SNPa; DNA-nutrient database.
- Tackle the impact of ncRNA, both from the individual and diet-derived ncRNA, in target organs and their impact on different pathologies (e.g. obesity, cancer). Study the impact of dietary interventions on miRNA and their potential as biomarkers/therapeutic tools for precision nutrition in specific pathologies.
- Systems biology/Bioinformatics - Integration of clinical data, genetic background, microbiota, and other multiple -omic data (epigenomic, transcriptomic, proteomic, metabolomic, metagenomics...) in clinical trials and cohort studies requires powerful bioinformatic resources, including machine-learning algorithms able to predict response based on data integration.

7.3.9 Reshaping microbiota through nutrition

Reshaping host-microbiota interactions through personalized nutrition would be a new tool for improving health towards disease control and prevention. It is important to understand the host response based on the microbiota profile in specific dietary intervention (e.g. responders and non-responders). When (circadian feeding patterns and intermittent fasting) and how (cooking processes) the specific dietary intake or food are consumed may exert a different impact on host physiology and microbiota, as well as in the host-microbiome interaction. To date, only limited research has been conducted to assess whether nutritional factors lead to changes in the microbiota and drive host-microbiota interactions mainly in the critical periods of life as early infancy and elderly. Such observations would explain the great interest in perinatal interventions and the potential use of probiotics, prebiotics and symbiotics to promote an "adequate microbiota" and, thus, to beneficially affect health. At the same time, there is significant interest in microbiota-related research aiming to establish the identity of specific microorganisms, microbial molecules and metabolites that contribute to the host's physiology, metabolisms and health. Understanding how the microbiome responds to dietary constituents and the subsequent biological impact, as well as clinical consequences, can be used for the development of precision-tailored dietary interventions.

- To investigate how specific dietary nutrients confers the organism with benefits that go beyond their nutritional input by helping to improve general well-being or reducing the risk of disease.
- Understand the host-microbiome-diet interactions and mechanisms behind.
- To identify specific microorganisms, microbial molecules and/or metabolites contributing to the host's physiology, metabolisms and health.
- To understand the host response based on microbiota profile in different specific dietary intervention (responders and non-responders).
- Determine to what extent human health is modulated by when (circadian feeding patterns and intermittent fasting) and how (cooking processes) dietary nutrients are consumed.
- Develop mechanistic predictive models of the effect of dietary components and microbiota-based products on health outcomes.

7.3.10 Towards a more global and mechanistic understanding of epigenetic consequences of physical exercise

There are several important questions that should be addressed in coming years regarding how epigenetic alterations due to physical activity can impact human health and disease:

- Design animal models to study the molecular mechanisms behind the beneficial or harmful effects of exercise that can be transferred to humans (e.g. in elite athletes).
- Implementation of new generation epigenetic technologies to study epigenetic marks at the genomewide level.
- Integration of epigenomic data with data obtained from other -omics (i.e. transcriptomics and proteomics) to identify epigenetic changes with functional effects.
- The study of the effect of exercise on the epigenome of different cell types and tissues to identify common and specific changes.

7.3.11 Defining the etiological role of epigenetic alterations induced by tobacco and alcohol in human disease

Most of the current findings of epigenetic alterations are the result of association studies. However, this approach has limited power in clarifying whether these changes are causes or consequences of alcohol or tobacco-associated pathology. The signaling routes and molecular mechanisms involved remain to be defined by the development of mechanistic studies. Moreover, because of its accessibility, the majority of epigenetic changes have been described in peripheral blood, which is not the primary target tissue of alcohol nor tobacco. It is probable that, aside from biomarkers, more functional associations of epigenetic alterations and genetic expression will be detected by examining other tissues more directly related to these compounds.

CHAPTER 7 REFERENCES

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