HHS Public Access

Author manuscript

JPEN J Parenter Enteral Nutr. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

JPEN J Parenter Enteral Nutr. 2019 July ; 43(5): 627–637. doi:10.1002/jpen.1536.

RW-2018 - Research Workshop: The Effect of Nutrition on Epigenetic Status, Growth, and Health

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Abstract

The goal of the 2018 ASPEN Research Workshop was to explore the influence of nutrition and dietary exposure to xenobiotics on the epigenome during critical periods in development and how these exposures influence both disease incidence and severity transgenerationally.

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The authors have nothing to disclose and have no conflicts of interest.

A growing compendium of research indicates that the incidence and severity of common and costly human diseases may be influenced by dietary exposures and deficiencies that modify the epigenome. The greatest periods of vulnerability to these exposures are the periconception period and early childhood. Xenobiotics in the food chain, protein malnutrition, and methyl donor deficiencies could have a profound bearing on the risk of developing heart disease, diabetes, obesity, hypertension and mental illness over multiple generations. The financial impact and the life burden of these diseases is enormous. These and other aspects of nutrition, epigenetics and health are explored in this research workshop.

A primer on epi-genetics and disease

A growing percentage of diseases are now recognized as being inherited or transgenerational. In fact a growing percentage of specific diseases have a transgenerational etiology (table 1)(1).

Genetics (alterations to the DNA sequence) account for only a fraction of these inherited conditions. The remainder appear to be influenced by the environment. This shift in the incidence of heritable risk in one generation happens without a change in the genetic code. Instead it arises from molecular factors and processes around the DNA that regulate genome activity independent of DNA sequence (i.e. epigenetics). These processes include DNA methylation, histone modification, chromatin structure, non-coding RNA and RNA methylation. Because these changes do not alter the code, they are described as epigenetic which literally means “above the genes.” The entirety of these processes on the genome is termed the epigenome.

One of the most sensitive periods to exposure leading to alterations in the epigenome is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming. Alterations to the epigenome (epimutations) during this period can become transgenerational (affecting subsequent generations not exposed), and are hypothesized to be due to a permanent (imprinted-like) altered DNA methylation of the germ-line. (2) This transgenerational epigenetic mechanism appears to involve the actions of environmental compounds or nutritional events (e.g. DNA methylation) which permanently alter the epigenetic programming of the germ line. This in turn alters which genes are expressed in the developing organs to induce disease susceptibility.

A variety of environmental compounds or xenobiotics (substances that are foreign to the body or to an ecological system) induce epi-mutations, resulting in transgenerational inheritance of disease. These compounds include fungicides (vinclozolin), plastics (BPA and phthalates), pesticides (DDT) (2, 3), as well as dioxin, hydrocarbons, and chemotherapeutic agents (1). The hypothesis that environmental factors can reprogram the germ line induce heritable disease is a new paradigm and challenges the established dogma that heritable diseases only have a genetic or DNA sequence based etiology. This hypothesis provides the molecular basis for the Developmental Origins of Health and Disease or DOHaD.

The DOHaD hypothesis could explain in part the significant increases in the incidence of certain heritable diseases within specific populations. These rates far exceed the predicted

incidences based on the well-established baseline rates of genetic mutations. Evidence supporting this epigenetic mode of transgenerational heritable disease has now been observed in every life form studied to date including, plants, insects, worms, fish, rodents, pigs and humans (4).

Take home points and future directions

Take home points: evidence increasingly suggests that alterations in methylation patterns, chromatin structure, histones, and mRNA, as opposed to changes in the genetic code, drastically impacts gene expression and influence susceptibility to heritable disease states. Further work needs to be done to understand the molecular mechanisms linking untoward exposures to untoward outcomes as well as adding to the list of known agents causing these events.

Using famines to study disease risk on the next generation

Living conditions during gestation (stress, poor nutrition, environmental exposure to xenobiotics) not only have an immediate impact on the well-being of the mother, but appear to have long-lasting health effects on the offspring. In general, studies linking poor nutrition in pregnancy to outcomes in offspring can be severely biased if key confounders are ignored and the nature of the exposure is poorly defined.

One strategy to overcome these biases and investigate the long-term impact of poor early nutrition in gestation has been through the analysis of man-made famines as quasi-experimental events. This allows for the unbiased comparison between individuals exposed to such conditions to unexposed individuals or “controls”. This approach can provide important information on the long-term health impact of gestation if 1) the population at risk is readily identified 2) the timing and degree of exposure is known, and 3) the adult health outcomes are well defined. The application of these principles have been illustrated by studies of men and women born during the Ukraine Holomodor famine of 1932–33 (5), the Dutch Hunger Winter famine of 1944–45 (6–9), and the Chinese Great Leap Forward famine of 1959–61 (10). These Famines are a key starting point in understanding the relationship between nutrition, epimutations during gestation and adult disease.

The most studied famine is the Dutch Hunger Winter (6–9) which came about because of a German transport embargo in response to a rail strike in support of advancing Allied troops during WWII. The affected geographical region was limited to Western Netherlands. The Hunger Winter ended with the surrender of the German forces in May 1945. During the Hunger Winter the total calories were rationed to less than 900 calories per person. Children conceived and exposed during the first 10 weeks of gestation to these conditions demonstrated significantly higher risks of obesity, glucose intolerance and serum triglyceride levels when compared to unexposed controls and siblings. The exposed individuals also had double the rate of type 2 diabetes compared to siblings and controls even when adjustments for weight, waist-circumference, or waist-to-hip ratio were made (6–9). Similar observations of a higher predisposition of type 2 diabetes have been made in individuals conceived during the Ukraine Holomodor famine (5). Mechanistically the loss of methylation as a result of famine exposure during early gestation had a significant impact on

the expression of one of the genes that is a critical regulator of the glucose metabolism and a marker for susceptibility to obesity and type 2 diabetes: IGF-II (8, 11). These and other changes in methylation patterns in individuals exposed to the hunger winter during the first trimester of gestation are consistent with diseases patterns observed later in life (11, 12).

Take home points and future directions

The findings from epidemiological studies from these famines suggest that maternal nutrition during critical windows in gestation has a long-term impact on offspring's health. And that exposure to large segments of the population can affect entire generations of people. Future areas of interest are in defining additional exposures that may carry similar risks to famines and once these risks are known, are their nutrition counter measures strategies that could be used by pregnant women in early gestation or those trying to conceive?

A compelling animal model of fetal programming

Research from the Dutch Hunger Winter and other famines indicates that the risk of non-communicable disease in adulthood is related to *in utero* experience and the quality of maternal nutrition during pregnancy. Given the growing body of evidence from human epidemiologic studies there is a clear need for animal models to test this concept directly.

The mouse model of maternal protein restriction is an outstanding investigatory tool for studying epigenetic changes and DOHaD. Numerous studies in this model have shown that despite the small number of cells in the early embryo, exposures to the environment through the placenta offer an opportunity for the embryo to alter its development to optimize its fitness for survival. This early maternal-embryonic dialogue allows both extra-embryonic (placental) and true embryonic (fetal) cell lineages to adapt accordingly.

In the mouse model, poor maternal nutrition (specifically reductions in maternal protein intake to 9% of the diet) has been shown to promote placental and yolk sac tissues to compensate and develop increased efficiency of nutrient delivery to the fetus (13, 14). Fetal tissues in turn regulate growth rate to match nutrient availability. The embryo and placenta sense changes in the maternal nutrient environment (maternal low protein diet) specifically low insulin and low levels of branch chain amino acid. Extra-embryonic alterations (placental efficiency) then occur, including increased proliferation, nutrient capture and transport (13, 14). Meanwhile the embryo increases its growth by modulating ribosome biogenesis (a fundamental mechanism of cellular growth and metabolism) through DNA methylation levels of the rDNA promoter (15). Endocytosis (or nutrient capture) in the primitive endoderm and visceral yolk sac are associated with epigenetic modifications to the Gata6 promoter (16). This developmental 'plasticity' aids survival and competitiveness of the offspring.

Mild changes in maternal protein intake and the periconceptual environment also result in significant changes in the phenotype and physiology of the offspring later in life that includes, elevated serum glucose, obesity, and hypertension (14, 17–21). These programming changes can be particularly adaptive in seeking protein sources in a hunter-

gatherer setting. A higher blood glucose level translates into more readily accessible energy. Storing more fat is advantageous if calories are in low supply. Hyperactivity may be advantageous when hunting as is the capacity to run a higher blood pressure when chasing prey. In a modern world of caloric abundance, however, these changes to optimize the fitness of offspring for foraging and gathering food become maladaptive in terms of disease. Spikes in non-communicable diseases ensue, including cardiovascular disease, metabolic syndrome, and mental illness.

Periconceptional developmental programming (i.e. embryonic developmental decisions) can also be influenced by a broad spectrum of conditions other than maternal nutrition. These include maternal age (19), xenobiotic exposures (1, 2) as well as conditions of exposure during *in vitro* fertilization and assisted reproductive technologies (ART) (22, 23). These have all been associated with increased long-term risk of cardio-metabolic dysfunction in human as well as in animal models. To minimize the risk of heritable non-communicable, non-genetic or DOHaD diseases there must be greater resources directed towards preconception care and preparation for pregnancy in order to protect the health of the next generation as well as those that follow.

Take home points and future directions

The mouse is an excellent high throughput model for understanding the Periconceptual/early gestation epigenetic mechanisms underpinning DoHaD. This lays a very clear empiric foundation in support of this hypothesis, and can serve a powerful screening tool to assess for epigenetic risk.

The agouti mouse

The viable yellow agouti (AVY) mouse is one of the most powerful tools to study epigenetics. This is because the coat color can vary from brown to yellow, and this color corresponds to susceptibility to disease states in adulthood. Brown mice are heathy, with low incidence of cancer, diabetes, and obesity whereas yellow colored mice develop all of these diseases in high frequencies. Agouti mice are an established mouse line that are inbred and genetically identical. Yet based on changes to the epigenome one can generate inbred, genetically identical mice where the phenotype varies widely based on what the mother ate during the early part of gestation.

The change in coat color is the result of a transposable element upstream of the agouti gene referred to as Avy locus. The color of the hair shaft and how the color is controlled is important to understanding the readout of this animal model. In normal mice, the base of the hair shaft is yellow and the remainder is black giving the mice their brown or agouti color. If the transposable element in the Avy mouse is methylated, or turned off, a brown animal is generated. If the transposable element is not methylated, then the gene is turned on everywhere, including in the animal's brain. The coat color is yellow and the agouti protein is inappropriately produced in the satiation center of the brain. There, the agouti protein binds to the melanocortin 4 receptor (Mc4r). As a result of this the animal doesn't know it is full, and it eats itself into obesity, diabetes, and cancer. Thus, the adult phenotypic read-out is established based on epigenetic changes very early in development, making the Avy

mouse a powerful tool to screen xenobiotics and other biological and physical agents for their impact on the epigenome (24). There is a human homolog for agouti, but relationships for disease in humans has not been shown.

Normally within a given litter, there is a distribution in coat color among the offspring. Giving the mother methyl donors in her diet (e.g. folate, B12, choline, etc.) methylates the transposable element in the agouti gene, and shifts the distribution of her offspring towards the heavily mottled or completely brown color (25).

Interestingly, there are other agents that are not methyl donors that can also shift the epigenetic profile of agouti mice. For example, genistein is a weak phytoestrogenic compound in soy that is considered to have many health benefits (26–29). It is not a methyl donor; however, supplementation of the maternal diet with genistein results in a change in the methylation pattern of the agouti gene, and a marked shift away from obesity and yellow coat color in offspring (30).

Endocrine disrupting agents such a bisphenol A (BPA) are non-genotoxic compounds that cause cancer without disrupting the genome (2, 25). At levels present in humans, BPA causes hypomethylation at the agouti locus in mice, and switches coat color distribution of the offspring towards yellow. Importantly, supplementing BPA-exposed pregnant dams with methyl donors or compounds such as genistein negates the effects of this environmental toxicant (30). There is a human homolog for agouti but relationships for disease in humans has not been shown.

It is not just nutrition and chemical compounds that can affect the epigenome. Physical agents can do it too. X-rays, gamma rays, and cosmic rays are forms of Ionizing radiation that can damage DNA either directly through the formation of ionizations or indirectly by the creation of reactive oxygen species (ROS) through the radiolysis of water. Approximately, 80% of radiation-induced biological damage is caused by the formation of ROS, which experimentally can be mitigated by antioxidants. The standard risk assessment curve for radiation that is currently being used by the EPA and other regulatory agencies is a linear no threshold (LNT) model which argues that there is no dose of radiation that doesn't cause problems. Thus, the LNT model predicts that exposure of Avy mice *in utero* to any dose of radiation would shift the distribution of coat color and disease profile in the offspring from brown and lean to yellow and obese. In contrast to this prediction, low doses of radiation actually have the opposite effect. Low dose exposure at or around implantation results in a significant excess of brown, lean animals and a decrease in yellow animals compared to unexposed offspring. Methylation levels at the Avy locus are also increased corresponding to these shifts in phenotypes. Additionally, antioxidant supplementation of the maternal diet returned the coat color distribution of the radiation-exposed offspring back to the control condition (31).

These results indicate that low doses of radiation produce a positive adaptive effects in the Avy mice (i.e. radiation hormesis) through the formation of free radicals, and that these benefits are negated with antioxidants. Questions that presently remain to be answered are: 1) what are the signal transduction pathways that link the formation of ROS to programming

of the epigenome; 2) does low dose ionizing radiation alter the human epigenome; 3) is the LNT dose response model inappropriate for estimating human risk to low doses of ionizing radiation; 4) can nutritional supplements negate epigenetically mediated deleterious effects of physical and chemical agents; and 5) in light on the untoward effects of anti-oxidants in the agouti mouse model, when are nutritional supplements actually harmful?

Take home points and future directions

The findings from this model findings unequivocally demonstrate that exposure of offspring to both physical and chemical agents while in the womb alters their adult disease susceptibility by modifying the epigenome. Consequently, it is no longer appropriate to view disease susceptibility as something that happens only in adulthood (32). However, demonstrating additional correlations between similar epigenetic exposures in human development and subsequent disease states is needed.

Focusing on the outliers

If there is a connection between prenatal exposures and epigenetic alterations, on the one hand, and epigenetic alterations and disease, on the other hand, then an all-important, but unstated assumption is that an individual's environmental exposure history must be recorded as epigenetic alterations in the cellular genomes of normal tissues. The existence of such a "molecular fossil record" of individual environmental exposures has the potential to be both diagnostic and prognostic in any disease in which gene/environment interactions are thought to be significant.

The consistent epidemiological associations between extremes of infant birth weight and both undesirable perinatal/infant outcomes and adult diseases indicates that prenatal nutrition plays a role in programming long-term health. Thus, DNA methylation can serve as a potential marker of undesirable environmental exposures by examining normal tissues in individuals subjected to such an exposure. Populations being studied using this approach include children exposed to unusual environments early in development (children conceived by assisted reproduction (22, 23)), children who are small for gestational age/low birth weight, as well as individuals with colorectal cancer because of the well-documented association between this disease and high fat diets (33, 34).

DNA methylation differences associated with both assisted reproduction and low birth weight have been found and validated in independent populations. However, the magnitude of these differences is small in comparison to population level variability. Careful analysis of such population level variability suggests that normal individuals differ strongly in their susceptibility to exposure-mediated epigenetic "disruption". This in turn suggests that true and measurable effects of environmental factors on epigenotype are most likely to be observed at the extremes of the intersection of environmental risk factors and human population variability (35). Careful analysis of such "outlier" populations is most likely to shed light on the molecular mechanisms by which suspected environmental risk factors are able to drive the epigenetic processes involved in disease risk. Consistent with the expectation that not all individuals are equally susceptible to individual environmental exposures or all environmental exposures are not equally disruptive to the epigenome (36,

37), such normal tissue epigenetic “outliers” are found among colorectal cancer patients examined in The Cancer Genome Atlas (TCGA) population (38).

By focusing on the outliers to understand the role of the epigenome in recording undesirable environmental exposures (and, especially, poor nutritional exposures) one may need tens of thousands of subjects to study because outliers comprise such a small fraction of the individuals in any disease phenotype of interest. However, focusing on the extremes of the exposures (i.e., famine or extreme obesity if we are considering nutrition as an exposure) might allow insight into the mechanisms by which the exposures mediate less grievous effects in the general population (35).

Take home points and future directions

The epigenome can potentially serve a record of an individual’s history of environmental exposures. To link this to disease and outcomes, we must identify “outlier” individuals and correlate their “outlier methylation signature” with specific environmental exposures. There after we must conduct longitudinal follow-up of children who have had pre-natal exposures to correlate there outlier methylation signatures with specific disease states.

How Genes and Environment Interact

It is impossible to discuss the role of epigenetics in disease without considering xenobiotics (substances that are foreign to the body or to an ecological system) in the food chain. In particular, the heavy metals warrant discussion because of their known deleterious biological effects, and the ability to detect and measure them in biological samples has improved dramatically with the introduction of mass spectrometry. It is now possible to measure 20 to 50 different chemicals in the human body with very small volumes of blood. Prior to mass spectrometry, levels were measured using very large volumes of blood. What is being discovered is that humans are in fact exposed to much higher levels of heavy metals than we were aware of previously. As an example, the average newborn has between 50 and 200 xenobiotics in their cord blood at birth. From these new technologies we have discovered that two of these xenobiotics, lead and cadmium, are found together in humans about 50% of the time (39). Neither of these elements are genotoxic yet they have a profound effect on gene expression. This has led to the hypothesis that both are modifying the epigenome. The molecular mechanism by which this occurs is actively being investigated and remains unknown. What also remains unknown is whether the epigenetic and biological effects that are observed in populations are due to exposure to lead alone or the combination of lead and cadmium.

Massive exposures and associated lead poisoning results in degenerative bone disease, cognitive impairment, reduced brain volume, and chronic kidney disease. Chelating agents (EDTA) are used to mitigate effects of poisoning, but are these approaches effective when much lower levels are present in the blood stream, especially in children where chelating agents may also chelate nutritive metals such as magnesium and iron? What is a safe exposure now that we have removed most of the major sources of poisoning? And what are the effects of low levels of cadmium which pairs with lead through numerous industrial legacies?

Unlike low doses of radiation which appear to stimulate hormesis in the agouti mouse model, lead and cadmium even at low levels of exposure are associated with lower birth weight, increased pre-term births and accelerated catchup growth (40, 41). *In vivo* and *in vitro* models indicate increased adipocyte numbers, and decreased insulin dependent glucose uptake. These are markers for metabolic syndrome (type 2 diabetes mellitus, hypertension, obesity, and cardiovascular disease). However, human clinical studies have been inconsistent in demonstrating a clear link between cardio-metabolic impairment/metabolic syndrome and cadmium exposure (40–42).

Unlike in the situations of massive exposures and poisonings, strategies to reduce the risk of low level exposures through supplementation with essential metals and nutrients such as iron, selenium, calcium and folate are of minimal benefit. Furthermore the presences of other micronutrients that are part of a multivitamin regimen such as Zinc, Copper, Magnesium and Manganese can increase serum levels of both cadmium and lead (41).

To study the effects of these xenobiotic exposures on the epigenome, the Newborn Epigenetics Study (NEST) was started. Risk factors included cigarette smoking, parental obesity, psychotropic medications, antibiotics, assisted reproductive therapies (ART), and organic and inorganic environmental chemicals. Phenotypic outcomes examined include birthweight, growth patterns, heart rate, pre-hypertension, obesity, cognition, attention deficit and temperament. To assess these exposures during early gestation, maternal erythrocytes were used to determine history of exposure during the first 3–4 months of pregnancy because erythrocytes have a life span of 120 days. Cadmium and lead both bind to the erythrocytes making this an excellent means of determining low level exposures as early as during conception. Women from Durham County, NC were then screened for lead and cadmium in their erythrocytes, and the addresses for these mothers were recorded as well. Levels were found that were above the upper end of what is actionable or reportable. There were three hot spots for lead exposure and one of those was also a hot spot for cadmium exposure. These geographic regions had overwhelmingly minority populations with an average annual income of less than $30,000. The primary source for these exposures was surprisingly house dust. Cadmium exposure was associated with lower birth weight, and increased obesity. Lead was associated with lower birth weight, accelerated growth in early childhood and pre-hypertension. These are all early indicators of cardio-metabolic syndrome in childhood (39, 40, 42).

So what do you do when you have association results? The possibility that confounding by difficult-to-measure, non-chemical stressors that co-occur with poverty and ethnic minority status, makes it very difficult to advocate for a policy to reduce exposure, since cause-and-effect is difficult to infer. So, you confirm this in a model system, such the zebrafish model, because it is transparent and accumulated lipid can be observed in real time. In zebrafish, cadmium, but not lead exposure early in gestation significantly increased fatty deposits, similar to what was observed in children (42). This led to the conclusion that cadmium is indeed an obesogen. Testing whether cadmium at these non-occupational levels is an obesogen, in an environment where competing, but difficult-to-measure non-chemical stressors are absent (zebrafish tank) has allowed policy advocates to recommend testing for not just lead, but also for cadmium, in prenatal and pediatric clinics.

Now that the relationship between early prenatal cadmium exposure and childhood obesity is likely causal, what are the mechanisms? Because cadmium is only mildly mutagenic, epigenetic mechanisms are thought to be the likely mechanism. The question then is, are there cadmium exposure dependent changes in the methylome (The set of nucleic acid methylation modifications in an organism's genome or in a particular cell) that can be definitively linked to obesity and/or other components of metabolic syndrome? In order to establish this link, it becomes necessary to assess methylation within specific cell types as methylation can vary dramatically across the 260 cell types in the human body. This is why averaging the level of methylation across all cell types is unrevealing. The specificity and accuracy increases if specific cell populations are targeted. When monocytes were targeted, a 14–15 fold increase in methylation was observed after cigarette smoke exposure—a major source of cadmium exposure among smokers (42). The issue then becomes what genes are examined for changes in methylation? And is there an existing methylation profile to make comparisons to, so that exposures can be linked to shifts in the methylation profile. Here if becomes important to examine imprinted genes because they will retain their methylation patterns even after the normal developmental phase of demethylation (43). Thus, if there is an exposure during the normal developmental phase of demethylation, then it will be easy to detect in these changes to the methylation pattern of these imprinted genes. Imprinted genes are also networked. This means they are part of a regulatory network that interact with each other through a series of molecular regulators to govern programmatic gene expression in the cell. Because of this networking, one can look at fewer gene regions and make more accurate predictions about methylation states. These imprinted genes are important in cancer, diabetes and adipogenesis. The most studied is the *IGF2/H19* imprinted domain in humans. The mono-allelic expression is controlled by at least two differentially methylated regions (DMR) for *IGF2* and the intergenic region upstream of *H19*. Aberrant DMR methylation with *H19* DMR is linked to cancers and obesity. Recently, a study from the University of Cincinnati demonstrated that exposure to lead resulted in much lower levels of methylation of *IGF2/H19* DMRs indicating an epigenetic link between lead exposure and disease risk (43).

Another imprinted region is the *DLK1/MEG3* imprinted domain (11). *MEG3* which is a tumor suppressor gene. The *MEG3* DMR maintains an active unmethylated status allowing expression of *MEG3*. Aberrant *MEG3* DMR methylation is associated with decreased transcription of *MEG3*. Predictably, aberrant *MEG3* DMR methylation is associated with multiple cancers (41).

Cadmium exposures were associated with increased methylation at the *MEG3* DMR, associations were stronger in males and in African Americans. Intriguingly, individuals with increased methylation of *MEG3* DMR geographically clustered to a region of Durham County, NC that also had high exposure levels to lead. Again this suggests a strong association between exposure to this dyad of xenobiotics, methylation and the potential for cardiometabolic syndrome and increased risk for cancer (41).

Take home points and future directions

Lead and cadmium appear to have a significant effect on the epigenome and subsequent adult disease states even a very low levels of exposure in the blood. In the future, developing custom platforms or assessment tools may one day provide screening for these early exposures and a means of determining and managing the future associated disease risk.

Epigenetics and Personalized Health

When it comes to health and medicine, one size does not fit all. Diets that work for some people are ineffective for others, and the same medicine may cause side effects in only certain patients. Yet, many of today's doctors still give dietary recommendations or prescribe therapies based on population averages. Public dietary advice is also based on population-based data. For example, since their inception in 1980, the Dietary Guidelines for Americans (DGA) have been recommending that carbohydrates should constitute 45–65% of our diet. These recommendations have resulted in a steady increase in the consumption of carbohydrates, especially refined grains and sugars, which over the past decades is paralleled by a striking increase in the proportion of overweight and obese people from 40% in 1980 to over 70 % today. According to these alarming trends 3 out of 4 Americans will be overweight or obese by 2020 while the associated medical costs are estimated to increase by $48 billion to $66 billion/year (44). While an increase in refined grains and sugars is one of the major drivers of these trends, the one-size-fits all advice to focus on carbohydrate, as a primary energy source for everyone may be part of the problem. As a matter of fact, recent studies in personalized nutrition indicate that individuals have a highly variable response to carbohydrates, and the same piece of bread can spike blood sugar twice as much in one person as in another (45). It is therefore imperative to develop personalized dietary recommendations.

Personalized health is a paradigm shift away from one-size-fits-all healthcare toward a new, targeted approach to health and disease. Its goal is to define what is "good health" for each patient through the use of multi-omics markers (genome, epigenome, exposome, metabolome, proteome and microbiome) together with other physiological parameters such as age, gender and lifestyle. This will in turn allow doctors to predict, prevent, and cure patients by precisely shifting the focus from reactive to proactive healthcare (46). Personalized nutrition is an important part of personalized health that aims to understand what makes us metabolically unique in order to direct personalized dietary recommendations (47). An area of intense research in personalized nutrition is nutritional genomics, which explores the two-way interaction between our genome and the nutrients in our food. One component of this two way interaction is nutrigenetics — the study of how our genes affect our response to nutrients. The second component is nutrigenomics — the study of how the nutrients in the food we eat affect gene expression through epigenetic modifications and transcriptomic changes.

The molecular basis of nutrigenetics is that genetic variation can affect the function of proteins that are targets of nutrients, and this in turn affects the way our bodies absorb, process, and excrete different nutrients. There are several examples of nutrigenetics-based food reactions. One of the oldest known examples of this is favism, which arises from a

genetic deficiency in glucose-6-phosphate dehydrogenase. Deficiency in this enzyme leads to hemolytic anemia from exposures to infection, stress and fava beans. Intolerance to gluten, lactose and the amino acid phenylalanine are other examples of nutrigenetic-based food intolerances. Today these conditions can be screened by genetic testing and managed through personalized nutrition plans that exclude the toxic nutrient. Nutrigenetics can affect not only food intolerances but also other aspects of our response to nutrients. For example: the way we use macronutrients such as carbohydrates, fats and proteins; the way we absorb certain micronutrients such as vitamin D, and omega-3 fatty acids; how our blood glucose, lipids and hormones respond to these nutrients; how hungry we are; our food preferences, for example whether we have a sweet tooth or not; and finally how all these factors affect our overall body weight and composition.

But nutrigenetics is only half of the coin in nutritional genomics. The other half is nutrigenomics- the study of how food affects gene expression through epigenetic and transcriptomic changes. Nutrigenomics really changes how we think about food from simply calories and nourishment to a paradigm where food is information, and is one of the most powerful signals to our genome. Unlike genetic sequence, epigenetic modifications are dynamically remodeled by environmental signals. Nutrients send signals to epigenetic enzymes that write, read or erase epigenetic marks from our DNA. There are two general categories of epigenetic marks: while those placed prior to birth tend to be permanent as if written in ink, those placed after birth are potentially reversible as if written in pencil. The diet can affect both.

There is intense research around the possible applications of nutrigenomics in precision nutrition. One emerging approach is the use of specific diet plans to target epigenetic changes in genes acting in specific disease pathways. For example, a Mediterranean diet downregulates inflammation genes, calorie restriction down-regulates aging pathways, and ketogenic diets down-regulate excitatory neurotransmitters in the brain, which play an important role in epileptic seizures. Another possible application of epigenetics in personalized health is the development of epigenetic biomarkers that can be used to predict individual responses to weight loss diets. A few clinical trials show that it is possible to measure DNA methylation at specific genes before a diet intervention and predict whether someone is going to lose weight on a certain diet or not. Strikingly, many of these DNA methylation (DNAm) biomarkers are located in proximity of metabolic or obesity-associated genes such as *leptin, TNFα, BDNF* and *NPY* (48).

Other studies show that methylation at specific genomic loci can predict the onset of metabolic disease later in life. Obesity, diabetes, and metabolic diseases are complex trait disorders that are affected by genetic variation, epigenetic and regulatory networks, and environmental factors, often intermingling with each other. The contribution of genetic variation to obesity and metabolic disease is estimated to be around 35% (49). This is relatively modest when compared with a trait like height, 80–90% of which is determined by genetic sequence variants. Although we have identified more than 200 genetic loci associated with polygenic obesity, these can explain less than 3% of the variation in body max index (BMI) between people, and family history is a stronger predictor of polygenic obesity (50).

An emerging hypothesis is that epigenetic factors might help explain some of the remaining variability in BMI between people by mediating the interaction between genetic factors and environmental factors. Indeed recent studies suggest that epigenetic markers, in particular DNAm biomarkers, are more strongly associated with BMI and obesity traits than genetic sequence variants (48, 51). This is in line with what we know about epigenetic memory — the ability of the epigenome to store a molecular memory of our lifestyle exposure. According to the current model, most obesity-associated DNAm is a consequence of increased BMI, but once established can affect weight loss response or predispose to obesity-related disease such as type-2 diabetes and cardiovascular disease through activation or silencing of metabolic and obesity-related genes or regulatory sequences. A very promising candidate DNAm biomarker for the prediction of metabolic disease is the diabetes related gene *ABCG1*, which is involved in insulin secretion. A CpG site (*cg06500161*) within the *ABCG1* locus becomes hyper-methylated as a consequence of increased BMI in over 10,000 people of European and Asian-Indian ethnicity and across different tissues including blood, adipocytes and liver (52). Most importantly, in further longitudinal analyses (n = 2664) baseline methylation of *ABCG1* predicted the onset of T2DM seven years later with higher predictive value than standard risk factors including obesity, fasting glucose and hemoglobin A1c (53). Strikingly, one other study found that this particular CpG of ABCG1 was differentially methylated in sperm cells from obese versus lean men, and reverted to lean-type levels after weight loss surgery (54).

The big question now is: Are we ready for personalized nutrition based on nutritional genomics? The answer is not yet. On one side, nutrigenetic variants have low power to predict our response to food, which is a complex trait. According to a recent model, most complex traits are not simply polygenic (directly affected by many “core” genes), but omnigenic, which means that they are affected by potentially thousands of peripheral genes interacting with core genes and spanning the entire genome (55). While these “peripheral” genes each have small effects, their combined impact far exceeds the contributions of the core genes themselves. For this reason, it is currently impossible to accurately predict complex traits such as nutrient response based on genetic-testing of core genes alone. On the other side, the implementation of nutrigenomics will require a much deeper understanding of the epigenome and the methylome and how these are affected by diet. Other required measures to moving forward include the standardization of data collection and management, the integration of omics technologies, and the computational analysis of big data.

Take home points and future directions

Perhaps the greatest challenge is educating health care professionals, policymakers, and patients on epigenetics and personalized health while getting them to abandon beliefs and practices around nutrition that lack evidence yet are continually being pushed by aggressive advertising from the food and nutritional supplement industry. Doctors and other healthcare providers will need to know more about genomics, understand how that information is relevant to disease treatment and prevention, and share this knowledge with patients in order to involve them in their own treatment decisions.

Operationalizing epigenetics in a healthcare system.

Epigenetics is a relatively young field. It is becoming clear that in the future, as the correlations between exposures and disease become clearer and the mechanisms linking them become better defined, healthcare systems will implement policies to reduce the risk of epigenetic exposures. Until that based on the current state of knowledge there are several strategies that could be employed now.

To begin with, healthcare systems should examine their waste for agents that carry significant epigenetic risk. Are they flushing such agents into the environment such as pharmaceuticals and various industrial chemicals? Are there alternatives to these chemicals that could be used in their operations that carry less epigenetic risk?

Another area to consider is food waste as well as food sourcing. Over 50% of the food in this country is thrown away. That excess is courtesy of herbicides and pesticides, known to carry significant epigenetic risk. Efforts to reduce food waste should be paired with consideration of where the food comes from and how it is generated. By sourcing food locally through organic and environmentally conscientious growers, a healthcare system can actively reduce the exposures of their community to epigenomic toxins.

Another opportunity is around reducing the toxicants generated from burning fuel (56, 57). This releases tons of particulate matter in the atmosphere. The most cost effective way to decrease heating fuel toxins is conservation. So does the institution leave its lights on all night? This is a very straight forward first step that will also save the institution money. As with food waste, it is also important to consider the source of the fuel. Is the institution burning coal that is getting shipped in from other parts of the country or is it using locally sourced, renewable energy with less epigenetic risks? These are important questions to ask because it will be easy to determine whether the answers align with the institutional values of keeping people healthy.

One of the greatest impacts a healthcare organization can have is in sharing information about successful initiatives with other like-minded healthcare organizations. This can have a multiplier effect with national implications. Also by establishing strategic partnerships, like-minded partners can function as a nationwide buying group, leveraging their power as consumers to choose products that are less epigenetically impactful. This strategy can also be used to retreat from investments in companies that generate epigenetically dangerous agents.

Take home points and future directions

Although epigenetics is a relatively young field, healthcare systems can employ strategies to try and limit the risk of epigenetic exposures they create within their communities. Furthermore, as knowledge around this discipline grows and more correlations are found between exposures and disease states, opportunities for further risk management and risk mitigation we present themselves and become part of community health strategies

Acknowledgments

Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number R13HD094514

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Table 1

Defect/Disease	percentage that are inherited
•Spermatogenic Defect	(>90%)
•Male infertility	(complete ~10%, severe 20%)
•Kidney disease	(~30–40%)
•Prostate disease	(~50%)
•Increase in mammary tumor formation	(~10–20%)
•Behavior (Mate Preference, Anxiety & Stress)	(>90%)
•Pre-eclampsia-like during late pregnancy	(~10%)
•Premature Ovarian Failure POF	(>90%)
•Ovarian Polycystic Ovarian Disease	(>90%)
•Female Premature Pubertal Onset	(>90%)
•Obesity	(~10–50%)