Journal of Developmental Origins of Health and Disease (2017), **8**(5), 513–519.
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doi:10.1017/S2040174417000733
Themed Issue: Australian Perspectives: Outcomes from the 2016 ANZ

REVIEW

Epigenetics and DOHaD: from basics to birth and beyond

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Developmental origins of health and disease (DOHaD) is the study of how the early life environment can impact the risk of chronic diseases from childhood to adulthood and the mechanisms involved. Epigenetic modifications such as DNA methylation, histone modifications and non-coding RNAs are involved in mediating how early life environment impacts later health. This review is a summary of the Epigenetics and DOHaD workshop held at the 2016 DOHaD Society of Australia and New Zealand Conference. Our extensive knowledge of how the early life environment impacts later risk for chronic disease would not have been possible without animal models. In this review we highlight some animal model examples that demonstrate how an adverse early life exposure results in epigenetic and gene expression changes that may contribute to increased risk of chronic disease later in life. Type 2 diabetes and cardiovascular disease are chronic diseases with an increasing incidence due to the increased number of children and adults that are obese. Epigenetic changes such as DNA methylation have been shown to be associated with metabolic health measures and potentially predict future metabolic health status. Although more difficult to elucidate in humans, recent studies suggest that DNA methylation may be one of the epigenetic mechanisms that mediates the effects of early life exposures on later life risk of obesity and obesity related diseases. Finally, we discuss the role of the microbiome and how it is a new player in developmental programming and mediating early life exposures on later risk of chronic disease.

Received 17 July 2017; Revised 15 August 2017; Accepted 16 August 2017; First published online 11 September 2017

Key words: animal models, diet, epigenetics, metabolic health, microbiome

Epigenetic mechanisms

There are numerous definitions of epigenetics including the study of modifications such as DNA methylation, histone modifications and non-coding RNAs, which result in altered gene regulation without changing the underlying DNA sequence. These modifications alter gene expression by altering the genome architecture and accessibility. The term 'epigenetics' was first coined in 1942 by Conrad Waddington.[1] As a developmental biologist, he coined the term to explain how different cell types arose throughout development from the one genome.[1] In other words, epigenetics was how one genotype could give rise to the different phenotypes.[1]

There are many examples of epigenetic modifications that impact on phenotype. One of these is the Agouti mouse model where epigenetic regulation of the *Agouti viable yellow* (A^{vy}) allele determines coat colour and predisposition to obesity and disease.[2] When the A^{vy} allele is expressed, the mice have a yellow coat colour and are obese and more susceptible to developing diabetes and cancer. However, when female Agouti mice are fed a high methyl supplemented diet (folic acid, vitamin B_{12}, choline, betaine) their offspring are brown in colour and healthy and this is associated with increased DNA methylation and silencing of A^{vy}.[3] The Agouti mouse has been a widely used model to show how the environment can impact epigenetics and hence phenotype.

Another example of an epigenetic phenomenon is monozygotic (identical) twins who do not share disease phenotypes, potentially due to their different environmental exposures resulting in changes in their epigenome.[4,5] Hence, an alternative definition for epigenetics are the mechanisms that result in phenotypic trait differences caused by environmental factors that impact on gene regulation.

Epigenetic modifications

Epigenetic modifications include DNA methylation, histone modifications and non-coding RNAs. Each of these epigenetic modifications can impact gene expression, however they are not independent of each other.[6] DNA methylation is the addition of a methyl group to a cytosine, predominantly in the context of a CpG but non-CpG DNA methylation is also an important regulator of gene expression.[7] DNA methylation at CpG rich promoters or gene regulatory regions is normally associated with gene repression,[7] however this is not always the case. DNA methylation results in an altered DNA structure and chromatin state and although DNA methylation is essential for gene regulation in some species, in others it is absent such as some species of yeast, fruit fly and worms.[8]

Histone modifications such as methylation, acetylation and phosphorylation, occur on lysine, arginine and serine residues

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https://doi.org/10.1017/S2040174417000733 Published online by Cambridge University Press

within histone tails.[9] The histone code refers to the combinations of these modifications which regulate the structure of the chromatin.[10] Certain combinations of histone modifications are characteristic of silenced or transcriptionally active chromatin.[10] For example, transcriptionally active genes are characterized by acetylation of lysine residues in the histone tails of histones H3 and H4.[10] Silenced genes or regions are characterized by trimethylation of lysine 27 (H3K27me3) and trimethylation of lysine 9 on histone H3 (H3K9me3).[10] There are a large number of histone modifications and hence a very large number of combinations which makes this a very complex system. There is epigenetic crosstalk between DNA methylation and histone modifications.[11]

Another epigenetic modification is non-coding RNA. These can be short, for example microRNAs (21–24nt), which can silence genes by base pairing with their targets resulting in mRNA degradation and/or inhibition of protein translation.[12] Long non-coding RNAs (lncRNA) are greater than 200nt and less is known about the function of these and how they regulate gene expression.[13] Non-coding RNAs also interact with DNA methylation and histone modifications resulting in a complex and multiple layers of gene regulation. Environmental and developmental changes can result in changes in any or all of these epigenetic modifications.

Epigenetics and developmental programming

Epigenetic mechanisms have long been described as the potential mechanism behind development programming in the context of DOHaD.[14,15] Developmental programming refers to how events early in life or during the development of a particular organ can programme that individual for an increased risk of chronic disease later in life.[14] Identification of the epigenetic changes that are involved in this phenomenon in humans is very difficult, mainly due to an inability to control their environmental exposures and access to core organs. However, animal studies provide the opportunity to study developmental programming and the epigenetic mechanisms involved.[16]

The importance of animal models

Animal models have been critical in demonstrating that there is a causal relationship between changes in the fetal environment and changes in the epigenotype. In addition to the ability to control for confounding factors (such as the current environment), animal models also allow the tissue specificity of effects to be determined. Whereas epigenetic studies of programming in humans are generally limited to clinically accessible tissues such as blood, the use of animal models enables assessment of more metabolically relevant tissues such as pancreatic islets and liver, giving more mechanistic insight into how changes in the epigenotype can impact on gene expression and consequently function.

Initial studies in animals focussed on changes in DNA methylation and this remains the most extensively studied epigenetic modification in a programming context. Studies have primarily adopted a candidate approach, and transcription factors have been studied as one of the most conceptually attractive class of targets. These can act as nodes of regulation, with one transcription factor having the ability to regulate expression of a network of genes. Therefore, by epigenetically dysregulating expression of a transcription factor, this will in turn lead to the coordinated dysregulation of a panel of genes the specific transcription factor regulates. One of the earliest studies of programmed epigenetic changes focussed on the transcription factor peroxisome proliferator-activated receptor alpha (*Ppara*). Lillycrop *et al.* demonstrated that maternal protein restriction in mice led to decreased DNA methylation of *Ppara* and an increase in the corresponding mRNA.[17] The glucocorticoid receptor (*Nr3c1*) is another DNA binding protein shown to be epigenetically regulated by the early environment. Using a rat model of maternal care (pup grooming and nursing), Weaver *et al.* showed that maternal behaviour during lactation impacted on the methylation of the glucocorticoid receptor in the hippocampus.[18] Developmental transcription factors also represent attractive targets of epigenetic programming as they are expressed early during development. Two important developmental transcription factors in the endocrine pancreas, PDX1 and Hepatic Nuclear Factor 4A (HNF4A) have been studied in detail. Using a rat model of intrauterine artery ligation (representing placental insufficiency), the Simmons laboratory demonstrated this led to increased methylation of the *Pdx1* promoter and an associated reduction in *Pdx1* mRNA expression in pancreatic islets.[19] Similarly the Ozanne laboratory demonstrated that maternal protein restriction led to increased methylation of the minimal promoter of *Hnf4a* in pancreatic islets and a parallel reduction in expression of the gene.[20] Promoter methylation of the locus increased with age and this was associated with a progressive reduction in gene expression.

Changes in histone modifications have been less extensively studied in a programming context. However, they do appear to be an important epigenetic target of programmed dysregulation. In the study of *Pdx1* described above,[19] programmed changes in histone modifications were present before changes in DNA methylation. Reduced acetylation of histone H3 and H4 was apparent from fetal life and the reduction got progressively greater as the animals aged to 6 months, at which time changes in DNA methylation were also apparent. In the study of *Hnf4a* described above,[20] at 3 months of age there were no differences in histone modifications in the promoter region; however, there was enrichment of the repressive histone modification H3K9me2 in the enhancer region of low protein offspring. Long-range chromatin interaction experiments demonstrated this was associated with reduced interaction between the enhancer and the promoter of this locus, consistent with the reduced mRNA expression of *Hnf4a* in the low protein offspring islets. This highlights the importance of including regulatory regions beyond promoter regions when studying epigenetic programming. This same study also demonstrated that maternal diet could modulate age-associated changes in the epigenotype. The age-associated accumulation

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of the repressive mark H3K27me3 was greater in both the enhancer and the minimal promoter of the *Hnf4a* locus in the low protein offspring. This may explain the age-associated development of impaired glucose tolerance in these offspring and demonstrates the importance of including multiple time points in programming studies.

MicroRNAs are a more recent focus of attention as potential targets of developmental programming. These are small non-coding RNA species that through binding sequence specifically to the 3' untranslated region of mRNA transcripts can either reduce mRNA stability and/or block translation. Like transcription factors they represent attractive nodes of regulation, as their epigenetic dysregulation will lead to coordinated dysregulation of the network of mRNAs that they regulate. Using the maternal protein restriction model, the Ozanne laboratory demonstrated that maternal diet led to increased expression of the imprinted miRNA, miR483 in adipose tissue.[21] *In vitro* studies demonstrated that over-expression of this miRNA could directly limit adipocyte lipid storage capacity. Limited lipid storage capacity in adipocytes leads to increased ectopic lipid deposition and development of insulin resistance. Importantly this study also demonstrated in human adipose tissue that low birth weight was associated with increased miR483 expression, suggesting this effect is conserved between humans and rodents. Studies using non-human primate model of maternal high fat feeding have shown that maternal diet also leads to programmed changes in miRNA expression in the heart.[22]

Epigenetics and metabolic health in humans

The increasing prevalence of obesity and related comorbidities, such as type 2 diabetes (T2DM) and cardiovascular disease, is a major public health problem. Our current obesogenic environment undoubtedly plays an important role in the obesity epidemic, but there is substantial variation in how people respond to the same environment. Obesity is a multifactorial condition that results from complex interactions between genetic and environmental factors and there is a growing interest in understanding the potential role of epigenetics as a mediator of these gene–environment interactions.

Initial evidence in support of a role of epigenetics in the development of obesity and T2DM was provided by animal studies but more recently, advances in epigenetic methodologies and the reduced cost of epigenome wide methylation analyses have led to a rapid expansion of studies in human populations. The vast majority of these human epigenome wide association studies (EWAS) focus on DNA methylation, and have revealed changes in methylation of specific genes in obese or T2DM individuals. For example, obesity, T2DM or related phenotypes such as hyperglycemia or increased waist circumference have been associated with DNA methylation changes in genes involved in metabolism and other processes (e.g. *HIF3A*, *SREBP1*, *CPT1A*, *ABCG1*, *TXNIP*, *PHOSPHO1*) in multiple cohorts and in different ethnicities.[23–30] A recent EWAS, the largest of its kind to date ($n = 10{,}261$), showed that body mass index (BMI) as a measure of obesity, is associated with widespread changes in DNA methylation in genes involved in metabolic and inflammatory pathways.[31]

In most human studies the methylation changes are measured when the obese or diabetic phenotype is already present, and it is therefore difficult to establish whether the observed DNA methylation changes are on the causal pathway to develop the disease or whether they are a consequence of the phenotype. Attempts have been made to establish causality by combining DNA methylation data with genetic data from the same individuals, using the Mendelian randomization approach.[32] Using this approach it was demonstrated that most of the identified obesity-associated changes in DNA methylation in adults were likely to be a consequence of the obese phenotype rather than the cause.[31] In line with this, it has been shown that weight loss in obese subjects can partly reverse obesity-associated DNA methylation changes back to normal levels.[33–35]

However, even if many of the identified DNA methylation changes might not be causal factors in obesity development, DNA methylation changes have been successful in predicting future risk for T2DM,[23,31] and as such may be useful markers to identify (obese) individuals at increased risk for metabolic health problems. There are also indications that some DNA methylation changes at birth or in early childhood are associated with adiposity in later life,[36,37] and increasing evidence is emerging that that the prenatal environment may affect the epigenome and influence risk of metabolic health problems.[38,39] Maternal energy and nutrient deficiencies as well as maternal overnutrition, obesity or diabetes have been shown to affect the offspring's epigenome.[39–42] Not only the *in utero* environment, but also the preconceptional environment, including the environment experienced by the father,[41,43] has been suggested to play a role in this developmental programming. While current findings indicate a role of epigenetics, human studies showing a direct link between the early exposure with both epigenetic changes in early life and subsequent disease risk are limited. Ideally longitudinal studies are needed that follow parents and their future offspring from pre-pregnancy to when they develop metabolic health issues.

Most of the current epigenome studies in humans have used easily accessible DNA from blood cells as their sample for methylation analyses. However, blood DNA methylation profiles may not necessarily reflect the situation in metabolic target tissues, and further work is needed to assess effects of the prenatal environment and obesity in other tissues than blood. At least for some of the identified metabolic health-related methylation changes there is a consistency in methylation patterns over multiple tissues such as adipose tissue, muscle, liver, brain and pancreas.[26,31,44] For example, Kuhnen *et al.* recently demonstrated that DNA methylation changes in proopiomelanocortin (*POMC*), a gene important for body weight regulation, in the brain of obese individuals were also reflected in blood and kidney.[44] Furthermore, they

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observed that *POMC* methylation levels were stable over time and associated with maternal nutrient status, suggesting an early life impact on methylation that is maintained throughout life.

Overall, it has been repeatedly demonstrated that epigenetic alterations are associated with measures of metabolic health, and even could be predictive of future metabolic health problems. Increasing evidence also points to the involvement of epigenetic mechanisms in mediating the effects of early life exposures on later life obesity and T2DM risk, but more longitudinal studies are needed to establish this two-way interaction between the prenatal environment, the epigenome and later life health, in humans.

Diet, microbiome and epigenetic variation: an emerging paradigm for DOHaD disease risk

Epigenetic marks can be influenced by genetic and environmental factors. Epigenetic changes of state often involve many different epigenetic marks in a coordinated manner. Changes to epigenetic state and gene expression go hand-in-hand. However, they are just two of many cellular components in each tissue of the body. Studying at least two cellular components has been termed 'systems biology' and it has been argued that such combinatorial approaches can better define disease mechanisms and lead to more accurate disease biomarkers.[45,46]

As recent research on the microbiota has accelerated, it has emerged that a two-way interaction exists between us, and the myriad microorganisms with which we share our body. From the day we are born, we influence the complexity of the microbiome and in turn it helps programme our immune system, metabolism and brain function. This relationship is mediated at least in part by epigenetics. As most of the advances in microbiome research have been with gut microbiota, the largest and most complex mammalian microbial community, we will focus on this niche. Knowledge gained in studies of these relationships will help us understand how gut dysbiosis originates, how it has knock-on effects throughout the body, and how we can use diet as a therapeutic agent to reverse dysbiosis and ill health.

Components of our diet can be metabolized by a diverse set of gut microbiota, which in turn, release metabolites into the gut lumen, many of which are physiologically active throughout the human body.[47–49] Some metabolites play a role in neural signalling by activating vagal nerve chemoreceptors, among other mechanisms.[50] Gut microbiota can also directly interact with enteric neurons. Gut epithelial cells (colonocytes) absorb host- and microbiota-generated metabolites and, in turn, these may influence gene expression through epigenetic mechanisms. Many metabolites pass into the bloodstream and can travel around the body and influence organ systems, including the immune system, in which they play a strong role in programming in early life.[47,51]

A two-way crosstalk between host epigenetic state and microbiota complexity

There are a growing number of examples of how microbiota can influence epigenetic state. Exposure of immature human colonic epithelial cells to a bacterial cocktail resulted in changes to DNA methylation in over 200 genomic regions.[52] Yu *et al.*, working in mice, concluded that the gut microbiome guides and/or facilitates postnatal epigenetic change in maturing intestinal stem cells.[53] Deep sequencing of human peripheral blood DNA methylomes in women revealed a clear association between bacterial predominance and methylation profile across the genome between women with high and low levels of the phylum Firmicutes.[54] However, this study could not say conclusively that one influenced the other. There are also a number of ways in which microbiota can influence epigenetic state by interfering with levels of epigenetic modifiers such as DNA methyltransferases and histone deacetylases (HDACs).[55,56] Such interactions are poorly understood but most likely involve direct interaction by microbial products including substrates for the epigenetic machinery.

It is known that polymorphisms in host genes involved in innate and adaptive immunity can influence microbiota complexity.[57–60] There have been few studies of how host epigenetic variants may influence microbial colonization in humans but there is no reason not to believe that epigenetic variants do not exist in similar genes. This idea is supported by a study in mice that showed a relationship between prenatal-administration of glucocorticoids, immediate postnatal epigenetic state in colonocytes and microbial complexity.[52] In a further mouse study, colonocyte DNA methylation at the promoter of the *Tlr4* gene, which functions in the innate immune system, was associated with microbiota complexity.[61,62] Furthermore, Liu *et al.* showed that in mice, host gut epithelial cells produce non-coding microRNAs that are released into the gut lumen, enter bacteria and regulate their expression and growth.[63] They also showed that fecal microRNAs are essential for the maintenance of normal gut microbiota. In summary, it is highly likely that a two-way crosstalk exists between host epigenetic state and microbiota complexity.

Short chain fatty acids as mediators of epigenetic change

A large number of bacterial metabolites have a documented epigenetic function.[48] Short chain fatty acids (SCFAs) including butyric, propionic and acetic acid are produced from the fermentation of complex carbohydrates by specific subsets of gut microbiota, particularly those found within the distal colon. [49] SCFAs are the main energy source for colonocytes and are understood to provide humans with up to 10% of their total daily energy requirements.[64] SCFAs also play essential roles in host immune regulation and the anti-inflammatory effects of SCFAs are considered to protect against obesity and metabolic syndrome.[65]

The mechanisms by which SCFAs exert their effects have started to be revealed. They can influence gene activity by functioning as HDAC inhibitors (HDACis), with the strongest

https://doi.org/10.1017/S2040174417000733 Published online by Cambridge University Press

effects seen with butyrate.[48] HDACis promote retention of acetylated histones, which are associated with gene activity. SCFAs can also induce changes in the methylation state of histones and DNA, which is likely to be aided by crosstalk between epigenetic marks. In a recent study of germ-free mice, gut microbial complexity and levels of histone acetylation and methylation in multiple tissues differed markedly between mice given an 'unhealthy' Western diet and those given a 'healthy' diet rich in polysaccharides.[66] Similar effects to the healthy diet were achieved using SCFAs alone, indicating their high potency in eliciting epigenetic change.

SCFAs are likely to elicit epigenetic change through their interaction with cellular free fatty-acid receptors (FFARs), a specific family of G-protein-coupled nutrient receptors.[49,64,65] FFARs are expressed on immune cells, colonocytes and white adipose tissue and help to regulate the host's immune system, as well as playing numerous roles in metabolism. In humans, *FFAR2* (also known as *GPR43*) is activated by acetate, propionate, and to some extent, butyrate; *FFAR3* (*GRP41*) is activated by butyrate and propionate, and *NIACR* (the niacin receptor or *GPR109A*) is activated by butyrate.[49,64,65]

Cellular levels of specific SCFAs have been associated with DNA methylation levels in a small but growing number of studies. Fecal levels of six SCFAs were associated with global DNA methylation in human rectal biopsies.[67] Remely *et al*. found a significant correlation between a higher BMI and lower methylation of *FFAR3* in peripheral blood but no association with global methylation measured using the proxy of the LINE1 interspersed repeat.[68] A well-designed study in mouse gut organoids (isolated colonic crypts) showed that two different human commensal bacteria induced very different changes in gene expression, and that such changes could be to some extent recapitulated using isolated SCFAs.[69] In conclusion, SCFAs are a major influence on epigenetic and transcriptional state in the gut and other tissues. Future studies will likely reveal the role of other microbial metabolites on host epigenetic state.

Epigenetics-based anti-microbial therapeutics

Knowledge of the two-way relationship between microbiota and epigenetics could be used to treat, and even prevent, disease. Based on findings from animal studies, there have been a number of human clinical trials involving diet probiotics for a wide variety of human disorders, from obesity to Alzheimer's disease and cancer.[70,71] The field is in its infancy but there have been promising results, although the beneficial effects may not remain after intervention ceases. The suggestion has also been made that such interventions will need to be tailored to an individual's pre-existing gut microbiome.[71]

Epigenetically active nutrients, sometimes referred to as nutraceuticals, have been found to be protective against multiple disorders including those of the gastrointestinal tract, cardiovascular disease and cancer.[72] Research into their mechanisms of action are in their infancy but it was shown recently that epigallocatechin gallate prevented high fat diet-induced changes to gut microbiota, host gene expression and DNA methylation.[73] Clearly, there is still a lot to be learned about how we can modify our microbiota to prevent disease. Recent advances have started to shed light on the role of epigenetics in bi-directional host-microbiota interactions. As evidence grows, we will be better placed to modulate our environment to optimize our personal gut microbiomes, to treat, or better, prevent chronic illness.

Conclusions and future perspectives

This review highlights some of the research topics that are part of the much larger DOHaD area of study and in particular has focussed on the epigenetic mechanisms involved. The role of epigenetics in developmental programming has been greatly advanced by the use of animal models, which has clearly identified how early life adversity impacts on health and disease later in life but also provided a more controlled system to dissect out the epigenetic mechanisms involved. Defining the epigenetic mechanisms involved in developmental programming in humans is more difficult and requires longitudinal studies. However, increasing evidence, at least for DNA methylation, suggest that it may mediate or be a marker of early life exposures and hence potential biomarkers for predicting individuals at an increased risk of disease later in life such as obesity and type 2 diabetes. More recently, it has been shown that the microbiome can alter epigenetic modifications so new models of developmental programming need to include the microbiome. Future research in this area requires large human longitudinal studies with bio-specimens and detailed cohort information so that we can understand the underlying epigenetic mechanisms involved in DOHaD. By doing so, we can use this epigenetic information as biomarkers for identifying individuals at increased risk and requiring early interventions.

Acknowledgments

JMC is supported by grants from the Australian National Health and Medical Research Council (grant numbers 1011070 and 1083779), the Financial Markets Foundation For Children (grant number 032-2007) and the Murdoch Childrens Research Institute, which is funded by the Victorian Government's Operational Infrastructure Support Program.

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https://doi.org/10.1017/S2040174417000733 Published online by Cambridge University Press