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Current Opinion in Genetics & Development

Insights from epigenetic studies on human health and evolution

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Epigenetic variation represents a unique aspect of human biological variation that can shed light on our evolutionary history as well as the etiology of human disease. DNA methylation is the most commonly studied type of epigenetic modification and can alter gene expression without changing the underlying DNA sequence. DNA methylation occurs throughout all living organisms although its function seems to have evolved from genome defense in fungi, bacteria and plants to a more complex role in gene regulation and cellular differentiation in animals. Human DNA methylation was originally studied in imprinting diseases and cancer, but more recently has been investigated as a mechanism to mediate the impact of environmental and psychosocial stressors on human health and disease.

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Current Opinion in Genetics & Development 2018, **53**:36–42

This review comes from a themed issue on **Genetics and human origin**

Edited by **Quintana-Murci** and **Brenna M Henn**

https://doi.org/10.1016/j.gde.2018.06.008

Epigenetics and human evolution

Epigenetic variation has emerged as the latest lens through which to study human evolution and disease. Epigenetic modifications can alter gene expression without changing the underlying DNA sequence and, thus, illuminate a different facet of evolution and adaptation compared to genetic variants [1]. With both genetic and epigenetic variation, one can investigate the evolution of the genome at a molecular level as well as adaptation at individual and population levels. However, genetic change occurs less frequently and is more stable in comparison to epigenetic change, which can occur in response to environmental stimuli experienced during an individual's lifetime. Epigenetic responses to environmental stressors may have evolved to provide rapid, short-term responses to changes in the environment while genetic changes provided long-term adaptations.

Both epigenetic and genetic variants influence our response to diverse stimuli, ranging from environmental toxins to diet to emotional distress, and those responses can impact our physical and mental health (Figure 1). Epigenetic variants may also be altered by environmental stimuli, in contrast to more stable genetic variants. Moreover, different stimuli may feedback on epigenetic variants in a cyclical manner highlighting the complexity of epigenetic variation and the human condition, for example, exposure to lead or other toxins may create epigenetic modifications that alter the expression of genes involved in cognitive functioning and then lead to increased risk of joblessness, poverty, and continued exposure to environmental toxins. Epigenetic response to biological and psychosocial factors, like those illustrated in Figure 1, may have evolved in humans as an adaptation to increasingly complex stressors that are not experienced in simpler organisms [2].

DNA methylation is one of the most studied types of epigenetic modifications and typically occurs at cytosines followed by guanosines, that is, CpG sites. In addition to environmental factors, DNA sequence variants can influence the level of methylation at nearby CpG sites; those genetic variants are called methylation quantitative trait loci, or meQTLs, and have been found to associate with certain complex phenotypes and diseases [e.g. 3]. DNA methylation is one of the main epigenetic factors that controls gene regulation in mammals and plays a critical role in cellular differentiation and reprogramming [4]. Originally, DNA methylation was found to 'silence' genes when methylation occurred in promoter regions. More recently, research has shown that gene expression can be either increased or decreased depending on the region of the gene and genome that is methylated as well as the developmental stage and transcriptional activity of the genome [5–7].

Multiple research groups have assayed DNA methylation in healthy human populations in order to better understand how methylation contributes to natural human variation and to demonstrate how methylation is influenced by both genetic variation (meQTLs and genetic ancestry) and environmental exposures [8–10]. DNA methylation and meQTLs show high population-specificity and associations with complex phenotypes, such as age, can be highly consistent across diverse populations

Figure 1

A wide range of biological and psychosocial factors, both positive and negative, influence our lives (illustrated in the photographs in the figure), including poverty and homelessness, environmental exposures to toxins or irradiation, physical or emotional trauma, psychiatric disorders such as depression or anxiety, our emotional moods, pregnancy and family, our neighborhood, and nutrition and physical activity. Genetic and epigenetic variants may influence how we respond to these factors and how they impact our physical and mental health. Furthermore, these physical/psychosocial factors and genetic/epigenetic variants may feedback on each other in a cyclical manner, for example, exposure to lead or other toxins may create epigenetic modifications that alter the expression of genes involved in cognitive functioning and then lead to increased risk of joblessness, poverty, and further exposure to environmental toxins.
Source: This figure was created by Buster O'Connor and is reproduced with permission from Ref. [55], © by Annual Reviews, http://www.annualreviews.org Ref. [55].

[11,12••,13]. Recently, Carja *et al.* [14••] produced the first worldwide map of human DNA methylation at CpG sites. They found good correlation between population-specific levels of genetic, epigenetic and mRNA variation but much stronger correlation between genetic and epigenetic divergences suggesting that DNA methylation evolves in a clock-like fashion, similar to genetic variation. Furthermore, they found far greater evolutionary

stability of DNA methylation in humans relative to plants, revealing the possibility of a different role for DNA methylation in humans. Methylation maps have also been reconstructed for our extinct relatives, Neanderthals and Denisovans, by exploiting the natural degradation of methylated and unmethylated cytosines into thymines and uracils, respectively [15]. Comparing ancient and modern methylation maps identified methylation differences that may underlie the anatomical differences between us and our extinct relatives. Comparison of the maps also revealed that genes with differentially methylated regions are almost twice as likely to be associated with disease phenotypes than genes without such regions, highlighting the role that methylation-driven gene regulation may play in species-level evolution. More than a third of the identified disease genes are involved in neurological and psychiatric disorders, perhaps hinting at the complex coordination and regulation necessary for pathways that include these genes to maintain functionality, particularly across species.

Compared to epigenetic variation, genetic variation is more stable over time and more constrained, for example, binary genetic changes versus 0–100% variation in methylation at a given site. Although we have a good understanding of how genetic variation is created, maintained, selected and inherited, we know much less about these characteristics in epigenetic variation. From developmental biology, we know that virtually all DNA methylation marks in the mammalian genome are erased during embryogenesis, that is, the epigenetic slate is wiped clean at birth to allow epigenetic reprogramming of the genome [16]. Many researchers are investigating how methylation marks are created in the developing fetus, and how new methylation marks may be created later in childhood and adulthood. A key question is when genetic information from the parents is used versus environmental information during the methylation of a new CpG site, that is, *de novo* methylation versus maintenance of existing methylation. Li *et al.* [17•] reported more methylation sites with an environmental component than methylation sites with a genetic component in humans, and they found that more variation in genome-wide methylation was explained by environmental factors than genetic factors, highlighting the important role of the environment in generating methylation marks. Furthermore, Van Baak *et al.* [18] found that DNA methylation was more similar between monozygotic twins than could be explained by the twins' genetic identity and that their epigenetic 'supersimilar' sites exhibited plasticity with respect to the intrauterine environment, providing support for the importance of the intrauterine environment when generating methylation marks in the developing fetus. Furthermore, there are low but detectable levels of methylation, that is, <10%, that remain in mouse sperm and egg germ cells [19], which would be consistent with a low level of transgenerational epigenetic inheritance. Transgenerational inheritance of epigenetic marks, particularly those that are environmentally-induced, is one of the most controversial aspects of epigenetic variation.

Epigenetics and human disease

Imprinting diseases and cancer

The impact of DNA methylation on human disease was first studied in imprinting diseases in which different diseases are caused by the same genetic defect, like Prader–Willi and Angelman syndromes. Genomic imprinting leads to differential expression of certain genes exclusively from maternal or paternal chromosomes and results in different diseases depending on which parent carries the genetic defect. Results from recent studies suggest that non-parent-of-origin effects and environmental impacts may be more influential in imprinting diseases than previously thought [20,21].

The widespread epigenetic dysregulation leading to cancer has also been well-studied. The effect of promoter region hypermethylation and silencing of tumor suppressor genes in the development of cancer is fairly straightforward, but the effect of genome-wide hypomethylation and widespread aberrant histone modifications, another type of epigenetic alteration, requires further investigation [22–24]. Most recently, Yamashito *et al.* [25••] provided the first estimate of the relative contributions of genetic and epigenetic alterations to two types of cancer with known environmental etiologies (smoking for esophageal cancer and chronic inflammation for gastric cancer). They found that accumulation of rare genetic mutations significantly increased the risk of esophageal cancer but not gastric cancer and that increased DNA methylation levels of marker genes increased the risk of both cancers. These results have clear relevance for precision cancer risk diagnosis.

Environmental exposures

Exposure to environmental toxins and the impact on health is thought to be mediated, at least partially, through epigenetic modifications. A diverse range of toxin exposures have been tested for their effect on DNA methylation. Smoking tobacco was found to significantly associate with DNA methylation at 1500 CpG sites in buccal cells and this epigenetic signature of smoking was shown to discriminate between normal and lung cancer tissue [26]. A more recent study of over 5000 genome sequences from 17 different smoking-related cancers found a much small number of associated CpG sites that only occurred in lung cancer [27]. Smoking while pregnant has also been shown to associate with DNA methylation at a small number of CpG sites in newborns [28–30], with some sites remaining associated even if the mother quit smoking at the start of her pregnancy [31]. Furthermore, altered methylation of a subset of sites after prenatal exposure to smoking persists throughout childhood and adolescence and into midlife [32–35].

Exposure to air pollution has also been shown to alter DNA methylation and gene expression, particularly hypomethylation-mediated transcriptional activation of genes involved in particulate matter-associated and lung-associated diseases [36]. Furthermore, early life exposure to neurotoxic metals, like lead, is implicated in the development of cognitive and neurobehavioral problems in children and prenatal exposure has been associated with increased methylation at the promoter for the glucocorticoid receptor gene, *NR3C1* [37]. The glucocorticoid receptor binds cortisol and other glucocorticoids and is involved in many functions including fetal development, and the stress and immune responses.

Social and behavioral epigenetics

The idea that an epigenetic signature may be created by psychosocial stressors, such as poverty, childhood abuse, drug addiction, and war trauma, is more controversial [38]. Nevertheless, the field of social and behavioral epigenetics has exploded since Szyf and Meaney's landmark study in 2004 that demonstrated an epigenetic signature of maternal nurturing behaviors in rat offspring at the *NR3C1* promoter [39]. The DNA methylation changes were reversible with cross-fostering and persisted into adulthood, suggesting that intervention after early life abuse or neglect might have a large impact on later life health and wellbeing.

Over the past ten years, a wealth of studies have reported associations between DNA methylation and a range of psychosocial stressors including childhood abuse, military deployment, natural disasters, and war trauma [40–45]. One of the first studies in humans found altered DNA methylation at *NR3C1* in the brains of suicide victims with a history of childhood abuse relative to suicide victims with no childhood abuse and non-suicide controls [41]. Our studies in the Democratic Republic of Congo on maternal war trauma, including sexual violence, have documented genome-wide changes in DNA methylation in new mothers and more targeted methylation changes in newborns in a subset of genes involved in the stress response and neural development [42,46,47•,48–50]. It is now fairly well accepted that social and behavioral stressors can modify DNA methylation, with most studies focused on genes like *NR3C1*, brain-derived neurotrophic factor (*BDNF*), the serotonin transporter (*SLC6A4*) and other genes involved in the stress response and neural development [51•].

Early life adversity and later life health

Early life experiences are thought to have a large impact on later life health, which is a concept that is articulated in the Developmental Origins of Health and Disease hypothesis [52,53]. The possibility of an epigenetic mechanism to mediate the impact of early life exposures, particularly psychosocial exposures, on later life health has been proposed [54–56]. Multiple studies have shown an epigenetic effect of socioeconomic status (SES), with childhood SES impacting adult methylation more than adult SES [57,58]. McDade *et al.* [59••] tested early childhood SES and absence of a parent, in addition to biological factors like exposure to animal feces and duration of exclusive breastfeeding, and found associations with methylation changes in genes involved in the inflammatory response and biomarkers for inflammation, suggesting that the methylation changes were not merely correlational, but could be functional.

Prenatal stress exposures may be even more impactful than childhood experiences due to the phenotypic plasticity and adaptive, or maladaptive, responses that characterize a developing fetus [60]. DNA methylation signatures of a wide range of prenatal exposures have been documented, including alcohol and opioid exposure, gestational diabetes, intimate partner violence, maternal age and diet, preterm birth, and war trauma [42–44,61–68,69•]. Prenatal exposure to maternal depressive symptoms has been widely studied because depression during pregnancy can lead to multiple negative outcomes for offspring, including increased risk of obstetric complications and later mental health problems. Multiple studies of prenatal maternal depression have found evidence of DNA methylation changes in candidate genes, such as *NR3C1* and *SLC6A4*, in cord blood, infant saliva and adult venous blood [reviewed in 70]. Many of these methylation changes were found in enhancers, highlighting the role of gene regulation in mediating the impact of prenatal exposures to psychosocial stressors. However, a recent review of 22 published studies of DNA methylation and prenatal exposure to maternal stress, depression and anxiety did not find good overlap in associated CpG sites reported across studies [71]. Replication of specific CpG sites may be especially difficult with DNA methylation studies because we are still learning how DNA methylation impacts gene expression, that is, must key sites be methylated in order to affect gene expression, or is there a critical number of sites that must be methylated, or is an average level of methylation necessary?

Future directions

There are still more questions than answers when it comes to human epigenetics. Since DNA methylation varies from tissue to tissue, an active area of inquiry is whether or not easily accessible tissues, like blood and saliva, accurately reflect the methylation response of biological processes that are thought to occur in less accessible tissues like the brain. A recent study of matched DNA samples from four brain regions and whole blood found that >50% of variance in DNA methylation was explained by tissue type [72]. The authors opine that epigenetic studies of blood samples will provide little insight into underlying pathological processes but may identify biomarkers of diseases that manifest in the brain. It is important to remember that methylation-determined

differences in gene expression are part of the cellular differentiation process, so we expect methylation differences to occur between different cell types. Additional study is needed to determine if peripheral tissues possess a DNA methylation signature of the studied stressor that rises above the expected tissue-specific differences in DNA methylation.

There are gaps in our understanding of the ways in which DNA methylation can impact gene expression and resultant phenotypes as well as a lack of consensus on how DNA methylation should be measured and analyzed. Horvath [73,74] developed a method to calculate DNA methylation age in a wide range of tissues. Increasingly, acceleration of epigenetic age is being used to assess the impact of various factors or stressors on DNAmethylation [75–77]. Despite the inclination to consider the replication of specific CpG sites across different studies as the gold standard, it is possible that this criterion is too narrow and that promising leads will be abandoned. At this point, we do not know which exposures will leave a detectable methylation signature, and which ones will not. As the field of human epigenetics continues to mature, it is important to study the impact of a wide range of stressors by assaying an increasingly comprehensive set of epigenetic modifications across the genome and to publish both positive and negative findings.

Continued study of diverse and healthy populations is essential to better understand the basis of natural epigenetic variation. These studies will help define the background level of natural variation above which associations with disease phenotypes or psychosocial stress exposures can be established. Furthermore, since epigenetic modifications are impacted by both genetic variants and environmental stimuli, the study of population-level epigenetic variation is extremely fertile ground to investigate the complex evolutionary history of humans.

Conflict of interest statement

Nothing declared.

Acknowledgements

Funding was provided by NSF grants BCS 1719866 and 1231264 and grants from the University of Florida (UF) Clinical and Translational Science Institute, UF College of Liberal Arts and Science, and a UF Research Opportunity Seed Fund award.

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