The Role of Epigenetics in the Developmental Origins of Health and Disease

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Abstract

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that the prenatal and early postnatal environments shape the future probability of physical and mental well-being and risk of disease. A wealth of epidemiologic data document associations among maternal and infant nutrition, stress, and other exposures, and risk of chronic disease in later life including cardiovascular disease, hypertension, type 2 diabetes mellitus, obesity, neuropsychiatric disorders, and cancer. Extensive data from animal models support the biological plausibility of the DOHaD hypothesis. While the mechanisms underlying these observations remain unresolved, the DOHaD model assumes developmental plasticity, which allows adaptive regulation of embryonic, fetal, and infant development in response to nutritional and environmental perturbations. Establishment of epigenetic regulation during embryonic, fetal, and early postnatal life coincides with vulnerable ontogenic periods and provides a potential mechanism for long-lasting responses to transient environmental stimuli. In this chapter, we review recent progress in the epigenetic epidemiology of DOHaD and describe emerging approaches aimed at elucidating causal links between early environment, induced epigenetic alterations, and human disease.

Abbreviations

BMI	body mass index
CoRSIV	correlated region of systemic interindividual (epigenetic) variation
CpG	cytosine-guanine dinucleotide
DMR	differentially methylated region
DOHaD	Developmental Origins of Health and Disease
EWAS	epigenome-wide association study
GWAS	genome-wide association study
HM450	Illumina human methylation 450 microarray
IAP	intracisternal A particle
IGF2	insulin-like growth factor 2
IUGR	intrauterine growth retardation
PACE	Pregnancy and Childhood Epigenetics consortium
SIV	systemic interindividual (epigenetic) variation
SPLS-DA	sparse partial least squares discriminate analysis

6.1 The Developmental Origins of Health and Disease

The search for the origins of chronic disease has shifted the focus toward the earliest phases of the life course. While classic epidemiology has targeted lifestyle patterns of adults at various ages, in recent decades the importance of early life for determining lifelong health has been increasingly recognized. Following the seminal work of Rose [1], Forsdahl [2], and Barker [3, 4], the period from conception to birth and the first few years of life are considered critical in influencing disease susceptibility throughout life. This shift in thinking and research gave birth to the "Developmental Origins of Health and Disease" (DOHaD) hypothesis.

Epidemiologic studies support the hypothesis that chronic diseases have their roots in early life. Barker's work linked low birthweight to a number of cardiovascular diseases (including ischemic heart disease), hypertension, cholesterol levels, stroke, and impaired glucose tolerance [4–8]. His findings have been confirmed by other groups in different populations [9–11]. Data from the Dutch Famine in 1944/45, when food rations dropped below 1000 kcal/day for six months, suggest an increased risk of obesity among offspring of mothers exposed to the famine during the first and second trimester [12], glucose intolerance if exposure peaked during late gestation [13], and schizophrenia if conception occurred during the famine [14]. Other maternal characteristics such as maternal weight and malnutrition also increase the risk of coronary heart disease in the offspring [15].

However, there is trouble at both ends of the birthweight spectrum. Like low birthweight, high birthweight is also associated with adult obesity [16]. Similarly, women are more likely to become obese in adulthood if their mother was obese prior to pregnancy and/or had very high or very low gestational weight gain [17]. Furthermore, gestational diabetes (associated with fetal macrosomia) increases the risk of childhood and adult obesity in the offspring [18]. High birthweight is also associated with an elevated risk of several cancers. Numerous epidemiologic studies support the association between high birthweight and increased risk of premenopausal breast cancer [19, 20]. In addition, high birthweight has been linked to childhood leukemia [21], childhood brain tumors [22], and testicular cancer [23].

6.2 DOHaD Mechanisms

The fetal origins hypothesis suggests that perturbations at a critical period of development induce persistent alterations with potentially lifelong consequences. These epidemiologic observations led Hales and Barker to suggest the "thrifty phenotype" hypothesis, which proposes that poor fetal nutrition and growth lead to metabolic reprogramming of glycemic metabolism [24]. This adaptive developmental plasticity allows the fetus to adjust to and survive adverse environments. According to this model, a limited supply of transplacental nutrients compels the fetus to channel nutrients to the most vital organs, namely brain and heart, at the expense of other organs, which may remain underdeveloped and compromised in growth and function [25]. Moreover, permanent insulin resistance may be induced

during development and reduce basal metabolic requirements; this permits survival under suboptimal prenatal and predicted postnatal conditions [26]. Indeed, environmental perturbations may have a long-lasting impact at times of greatest plasticity during growth and development, while decreasing plasticity with increasing age allows less adaptation.

The potential benefits of such so-called predictive adaptive responses depend on the accuracy of the prediction; the cost of inaccurate predictions is high [26]. If developmental conditions that induce intrauterine growth retardation are followed by a resource-rich postnatal environment, high plasma glucose levels will coincide with insulin resistance, greatly increasing the risk for metabolic disease in later life [26]. This "mismatch" between predicted and actual postnatal environment may explain profound long-lasting implications for chronic disease among individuals prenatally exposed to the Dutch Hunger Winter, which lasted only nine months and was followed by normal nutritional availability [27]. Individual variation in sensitivity to mismatch and consequent disease susceptibility is likely due to a variety of factors including genetic variation and the degree of developmental plasticity [25].

6.3 Potential Critical Periods for Developmental Epigenetics

The biologic mechanisms underlying the long-term persistence of DOHaD phenomena are not well understood. Developmental plasticity allows a specific genotype to create alternative phenotypes depending on embryonic, intrauterine, and early postnatal conditions, which may induce lasting changes in chronic disease susceptibility. Among the various potential biologic mechanisms underlying developmental plasticity [28], environmental influences on developmental epigenetics are receiving increasing attention [29]. Epigenetics describes the study of mitotically heritable alterations in gene expression potential that are not mediated by DNA sequence alterations [30]. Essentially, epigenetic regulation involves a repertoire of cell-autonomous molecular modifications that govern selective access to the genetic information; because these are mitotically heritable, they are perpetuated in differentiated tissues. The specific molecular mechanisms that function interactively to heritably regulate chromatin conformation include DNA methylation (which occurs predominantly at cytosines within cytosine-guanine dinucleotides, i.e., CpG sites), various modifications of the histone proteins that package DNA in the nucleus, and autoregulatory DNA binding proteins [31]. The ontogenic periods during which these mechanisms undergo establishment and maturation suggest potential critical periods of environmental sensitivity (Fig. 6.1).

Many studies of epigenetics in DOHaD have focused on genomically imprinted genes. Genomic imprinting is the epigenetic silencing of either the maternal or paternal allele of specific genes by DNA methylation, leading to parent-of-origin-specific expression. Loss of imprinting results in the aberrant biallelic expression of an imprinted gene. Loss of imprinting of fetal growth genes, in particular that encoding insulin-like growth factor 2 (*IGF2*), has been associated with childhood disorders such as Beckwith–Wiedemann syndrome [32, 33], Silver–Russell

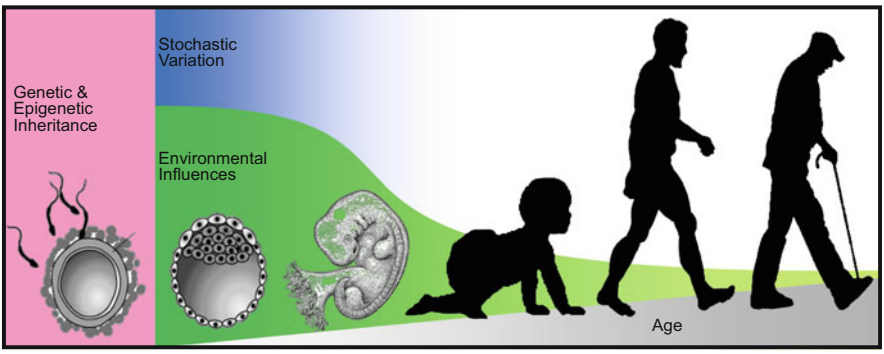

Fig. 6.1 Sources of interindividual variation in the epigenome. Environmental influences on the epigenome are likely most important during establishment of the epigenetic marks in prenatal and early postnatal development. [Reprinted with permission from R. A. Waterland and K. B. Michels: *Annu Rev Nutr* 27:363–388, 2007 [31]]

syndrome [34], and Wilms' tumor [35, 36], as well as with adult-onset diseases [37, 38]. In humans, approximately 100 imprinted genes have been identified. Since most imprinted genes play a role in intrauterine and early life growth, they have long been proposed as good candidates to translate early nutritional and environmental influences into fetal development [29]. Whether epigenetic regulation at imprinted genes is particularly susceptible to early developmental influences remains unresolved [39, 40].

Importantly, the epigenome is established at crucial developmental time points that coincide with vulnerable periods of adaptive plasticity. In the mouse model, each generation undergoes two waves of epigenomic reprogramming. As part of gametogenesis during mid-gestation development, primordial germ cells differentiate into oocyte and sperm [41], assuming distinct epigenomic profiles markedly different from those of somatic tissues. Then, after fertilization, the non-imprinted gene regions in the zygotic genome undergo another round of epigenetic reprogramming that restores totipotency. Genome-wide de novo methylation in the preimplantation embryo [42–45] permits cell fate commitment of the first cell lineages (discussed in more detail in Chap. 5). These dramatic waves of epigenetic reprogramming make mid-gestation and early embryonic development likely critical periods during which nutritional, environmental, and metabolic factors may affect the developmental establishment of epigenetic regulation in the gametes and somatic tissues, respectively.

As a first step toward understanding the role of epigenetic mechanisms in DOHaD, defining the window of susceptibility is crucial. In the mouse, for example, de novo methylation occurs at different times for imprinted and non-imprinted genes and in the developing female and male germline [43] (discussed in more detail in Chap. 5). The DNA methylation signature of non-imprinted genes may be most amenable to environmental stimuli just prior to implantation, when the totipotent blastocyst, largely stripped of genomic methylation, undergoes lineage-specific

remethylation during cellular differentiation. Nutritional and metabolic factors affecting the blastocyst during the early part of the first trimester therefore have great potential to augment or impair the introduction of cytosine methylation. The timing of remethylation of imprinted genes is less clear. By extrapolation from the mouse model, cytosine methylation of the differentially methylated regions (DMRs) of one of the two parental chromosomes is established at different time points for different imprinted genes [46–48]. In the mouse, maternal imprints are established at some point between oocyte development and ovulation [48], and paternal imprints are completed by the time spermatocytes enter meiosis [49]. Whether the establishment of imprinting marks is similar in humans remains to be established.

Periconceptional environmental stressors may yield downstream epigenetic effects in multiple tissues if induced epimutations are maintained during subsequent differentiation; perturbations during late gestation, on the other hand, are more likely to induce cell type-specific epigenetic changes [31]. Further, epigenetic development is not limited to prenatal life; for example, the early postnatal period appears to be a critical period for establishment of DNA methylation in the brain [50].

We have previously proposed two mechanisms to explain environmental influences on the developmental establishment of DNA methylation [31]. First, an imbalance in dietary methyl donors and/or activity of DNA methyltransferases may induce hyper- or hypomethylation. While most transposable elements in the mammalian genome are silenced by CpG methylation [51, 52], some are metastable and can also affect expression of neighboring genes [53]. Such metastable epialleles show large interindividual differences in DNA methylation and gene regulation—even among isogenic individuals—and appear particularly labile in response to environmental stimuli during developmental establishment of the epigenome.

Second, nutritional or environmental stimuli may alter transcriptional activity during periods of de novo DNA methylation, which may permanently alter epigenetic regulation and corresponding phenotypes. Genes actively transcribed during de novo methylation are protected from methylation and remain hypomethylated [54]. Interference with active transcription renders these promoters susceptible to de novo hypermethylation and alters their function [55].

The placenta's critical role in nutrient transfer from mother to fetus makes it particularly vulnerable to adverse intrauterine conditions. Whereas induced epigenetic changes in the soma persist to influence later phenotype, maternal nutrition may also induce epigenetic changes in the placenta, affecting nutrient transport and fetal growth [25]. Imprinted genes are highly expressed in the placenta, which may make them vulnerable to variation in maternal nutrition [56, 57].

6.4 First Clues from the Agouti Mouse

Seminal experiments in the agouti viable yellow (A^{vy}) mouse model support the idea that maternal nutrition can induce developmental programming via epigenetic mechanisms [58]. The *agouti* gene codes for yellow pigment in fur. Transposition of an IAP retrotransposon upstream of *agouti* resulted in the A^{vy} metastable epiallele.

DNA methylation of the retrotransposon exhibits spontaneous interindividual variation, controlling expression of the *agouti* gene and therefore the coat color of the animal. Moreover, supplementation of mouse dams during pregnancy with the dietary methyl donors and cofactors folic acid, vitamin B12, betaine, and choline shifts the coat color distribution of the offspring from yellow to brown [29, 59]. This was shown to occur by induced hypermethylation at the A^{vy} locus [58] systemically and permanently reducing expression of *agouti*.

Similarly, supplementation of the dams with the phytoestrogen genistein results in an analogous coat color shift also mediated through A^{vy} hypermethylation [60]. Maternal methyl donor supplementation studies in another murine metastable epiallele model, the *axin fused* mouse, corroborated the findings in the A^{vy} model [61], indicating that epigenetic regulation at metastable epialleles is generally susceptible to early environmental influences. Putative metastable epialleles are now being identified in humans [62]; as in the mouse models, these human loci show dramatic and systemic interindividual epigenetic variation that is influenced by maternal nutrition around the time of conception [63].

6.5 Epigenetic Epidemiology of DOHaD

Over the past two decades, numerous epidemiologic studies have been performed to explore the role of epigenetics in DOHaD. Within the epigenetic toolbox, DNA methylation is the most likely candidate to explain DOHaD observations due to its relative stability over time; furthermore, it is easiest to study due to its persistence within stored samples. Within the framework of DOHaD, epigenetic studies have addressed either the link between perinatal exposures and DNA methylation at various timepoints throughout life or the link between DNA methylation in early life and later health outcomes [64]. Studies exploring DNA methylation as a mediator connecting early life exposures and later life disease have been sparse [64]. In the following, we highlight some of the studies of particular interest.

Associations between prenatal and early life exposures and DNA methylation are being extensively examined by the Pregnancy and Childhood Epigenetics (PACE) consortium [65] as well as other groups. The best-established association is between maternal smoking during pregnancy and DNA methylation in the offspring cord blood, with a consistent change across most studies found in a CpG in the AHRR gene [66]. Four other CpGs that were changed in cord blood also showed changes in the placenta in a subset of these cohorts that had also collected placenta tissue [67]. In another PACE meta-analysis including 9340 mother–newborn dyads, both very high and very low maternal pre-pregnancy body mass index were linked to several small DNA methylation differences ($<0.2\%$ per BMI unit) in cord blood [68]. Interestingly, maternal alcohol consumption was not found to be associated with offspring cord blood methylation [69]. The PACE consortium and other cohorts have also linked preeclampsia and gestational diabetes to cord blood and placental DNA methylation [70–73].

Maternal self-reported high folic acid supplementation (defined as an average 1200 μg/day or more) was associated with 2.4% lower methylation at the *H19* differentially methylated region (DMR) in umbilical cord blood leukocytes if initiated before pregnancy and 3.7% less methylation at that locus if initiated during pregnancy, compared to mothers not reporting supplementation; no difference was found for the *IGF2* DMR0 [74]. A study from the Netherlands reported 4% higher methylation at one CpG of *IGF2* DMR0 in the blood of 17-month-old children whose mothers reported taking 400 μg folic acid during pregnancy compared to those whose mothers took no folic acid supplements; however, *IGF2* expression levels were not examined [75]. Maternal plasma folate levels during pregnancy were associated with DNA methylation in the cord blood of 1988 newborns [76].

In addition to smoking and folic acid supplementation, studies have linked other intrauterine exposures to DNA methylation in adulthood. Data on survivors of the Dutch Famine suggest that, compared to their unexposed siblings, individuals prenatally exposed to famine had somewhat lower DNA methylation at *IGF2* six decades later [77]. In this study of 60 same-sex sibling pairs, the authors examined five CpGs in the *IGF2* DMR0 and found, on average, 2.7% lower methylation among individuals exposed to famine in utero. Whether this small difference in methylation has any functional consequence remains unclear, in particular since the authors did not examine *IGF2* expression levels. Methylation differences of even smaller magnitude were observed for some other genes including *IL10, GNASAS, INSIGF, LEP*, and *MEG3* [78]. The association between an epigenetic difference assessed in adulthood and a prenatal exposure does not allow causal inference about the induction of that change by the prenatal factor, unless the change is already present directly after the exposure period [28, 31]. Of course, collecting appropriate samples in humans to test such causal pathways is logistically challenging.

Studies on DNA methylation in cord blood or placenta have considered several aspects of weight. A number of studies linked DNA methylation with birthweight with varying results [79–82]; in any event, changes observed did not persist to adulthood. The PACE meta-analyses of EWAS including 8825 neonates from 24 birth cohorts found birthweight associated with DNA methylation in neonatal blood at 914 sites, with a difference in birthweight ranging from −183 to +178 grams per 10% increase in methylation levels [83]. Some studies specifically explored the epigenetic profile of newborns with low birthweight or intrauterine growth retardation (IUGR). Einstein and colleagues compared cord blood samples from five IUGR and five normal pregnancies and identified methylation differences at a restricted number of loci [84]. A few small studies identified differences in methylation or expression of selected imprinted genes in the placenta and cord blood of IUGR or low birthweight compared to normal-weight infants [85–88] and in selected non-imprinted genes [89–91]. Conversely, high birthweight has been associated with increased promoter methylation of the glucocorticoid receptor gene in human placenta [84–88, 92]. Overall, differences in methylation in these studies were small, and it remains unclear whether DNA methylation changes are a cause or consequence of aberrant birthweight. Studies on childhood weight suggested an association between changes in newborn methylation of the *RXRA*

gene and the promoter or the long noncoding RNA *ANRIL* with childhood adiposity [93, 94]. CpG methylation of 68 CpGs in five candidate genes was assessed in umbilical cord tissue from healthy neonates in two prospective cohorts [93]; DNA methylation of one CpG was consistently associated with adiposity at the age of 9 in both cohorts.

In another childhood obesity study applying an array-based genome-scale screen to neonatal blood screening cards, although no statistically significant site emerged comparing the lowest and the highest BMI quartile at age 5, 13 CpG sites showed a $> 5\%$ difference in in DNA methylation levels [95]. All 13 were located in close proximity to the *nc886* gene. This gene, which encodes a small non-coding RNA, shows polymorphic imprinting in neonatal blood which appears to be modifiable by maternal age and nutrition status during pregnancy [40]. Methylation of the differentially methylated region nc886 may operate as a mediator between maternal characteristics and childhood outcomes, although a study demonstrating this link directly remains to be conducted.

Few studies have directly evaluated DNA methylation as a mediator between perinatal exposures and subsequent health outcomes. Cardenas et al. examined whether DNA methylation changes may mediate the association between intrauterine exposure to mercury and lower cognitive performance in childhood [96]. In newborn cord blood of 321 children, they found prenatal mercury levels were associated with lower DNA methylation at the paraoxonase 1 gene, which predicted lower regional cognitive test scores during early childhood. DNA methylation levels at this site, however, were attenuated in blood samples collected in mid-childhood, arguing against direct mediation.

Recently, focus has shifted to studies at the interface between epigenetics and the microbiome to explain DOHaD effect persistence [97]. Similar to epigenetic marks, the gut microbiome is established at birth, but remains malleable to a certain extent by lifestyle factors. The gut microbiome can influence DNA methylation and the activity of DNA methyltransferases and histone deacetylases, although the direction of this crosstalk is not always clear [98–101]. Bacterial metabolites, in particular short chain fatty acids, can function as HDAC inhibitors [102] and correlate with DNA methylation [103].

In summary, epigenetic mechanisms are likely candidates to explain at least some DOHaD phenomena. Nevertheless, despite a recent proliferation of studies in this area, it remains unclear whether the mostly small differences in DNA methylation at birth associated with intrauterine exposures have functional relevance and are maintained into adulthood. Whether the embryonic, intrauterine, and early postnatal environments affect adult disease susceptibility in humans via induced epigenetic alterations remains to be established. Although challenging, longitudinal cohorts assessing links between the periconceptional, pregnancy, and infant environment with adult health and disease status (including measurements of DNA methylation and potentially the gut microbiome at birth and throughout life) are needed to shed more light on these questions. Due to both their malleability by early nutrition and other lifestyle factors, and their noted long-term stability once established, DNA methylation and the gut microbiome hold promise for life-course prevention efforts.

6.6 Challenges for Epigenetic Epidemiology in DOHaD

In 2003 the International HapMap Project set out to identify common sequence variants in the human genome [104]. This "toolbox" enabled large-scale studies to test for associations between these variants and human diseases and phenotypes, heralding the dawn of the genome-wide association study (GWAS) era. In the past two decades, GWASs have identified an impressive and growing number of disease risk-associated genetic variants. Despite this success, however, the majority of individual variance in disease risk remains unexplained, contributing to increased interest in the idea that epigenetic variation could influence the etiology of disease [105–108] and leading to the development of so-called epigenome-wide association studies (EWAS) [109].

But the epigeneticists skipped a crucial step: no "epiHapMap" project was conducted. Rather, the overwhelming majority of the hundreds of "EWAS" studies in the literature employ DNA methylation arrays produced by Illumina (most notably the HM450 and more recently the EPIC850 array). Inexplicably, interindividual variation in DNA methylation was never considered in the design of the Illumina arrays [110, 111]. In fact, most of the probes on these arrays show negligible interindividual variation [112, 113]. A study evaluating the HM450 array in blood, using 256 technical replicates from 130 participants, showed that fewer than half of the CpG sites demonstrated greater interindividual variation than the variation due to technical errors [114]. Another study showed that the power of EWASs could be improved by focusing on the minority of CpG sites with substantial interindividual variation in DNA methylation [115]. A more recent study reported that in peripheral blood DNA, the greatest source of variation at most HM450 probes is intra-individual variability (most likely from variation in leukocyte composition) rather than interindividual variation [116]. The upgrade from the HM450 to the EPIC array in 2016 has not substantially improved the situation. Between HM450 and EPIC arrays, about 55% of the CpG sites show a correlation <0.20, due to low interindividual variability [117]. A recent study that used the EPIC array to examine test-retest reproducibility of peripheral blood DNA methylation of the same women over a one-year period [113] found extremely poor performance (average intraclass correlation coefficient of 0.22), and attributed this to the fact that "99.9% of CpG sites (covered by the array) in the non-sex chromosomes had similar methylation profiles between individuals." These data underscore the unfortunate fact that, over the last decade, over 1000 studies attempting to associate individual epigenetic variation with risk of disease have focused on genomic regions in which DNA methylation is largely invariant.

Another major factor overlooked by the HM450 and EPIC platforms is the cell type specificity of DNA methylation. Generally, we cannot "epigenotype" an individual using peripheral tissues such as blood; epigenetic variation detected in the blood may not be relevant for a disease involving the brain, for example. Reverse causality is another major confounding factor for epigenetic epidemiological studies. Even if the tissue of interest is obtained [118, 119], the disease process itself can

cause epigenetic differences, making it difficult to infer causality. Based on these observations, the designs of HM450 and EPIC arrays are far from ideal.

6.7 The Field Needs to Focus on Systemic Interindividual Epigenetic Variation

A logical approach to overcome these challenges is to focus on genomic regions displaying systemic interindividual variation (SIV) in DNA methylation [62, 120, 121]. A recent study, which could be viewed as a "mini-epiHapMap" project, conducted the largest screening of SIV regions in the human genome [122]. A computational algorithm was developed to analyze deep whole-genome bisulfite-sequencing data on tissues representing all three embryonic germ layers (thyroid, heart, and brain) from each of ten donors from the NIH Genotype-Tissue Expression project [123]. The authors identified 9926 correlated regions of systemic interindividual variation (CoRSIVs). Each CoRSIV is statistically significant ($P < 0.05$), includes at least 5 CpGs, and exhibits an interindividual methylation range of at least 20%. The multiple-tissue interindividual screening approach to identify SIV is similar to that previously used to identify candidate metastable epialleles [62, 120, 124], but unlike metastable epialleles, CoRSIVs are defined without regard to potential genetic influences on their interindividual variation.

Although only <1% of HM450 or EPIC probes are within CoRSIVs, these regions are often associated with a wide range of diseases. For example, the SIV region encompassing *nc886* (also known as *VTRNA2–1*) is a confirmed metastable epiallele; DNA methylation at this locus is influenced by maternal nutrition during periconceptional development [120]. More recently, evidence has emerged demonstrating this region is influenced by maternal alcohol use prior to pregnancy [40]. Additional studies found that methylation in this region is associated with risk of cancer [124, 125], type 2 diabetes [126], and preterm birth [127]. As mentioned above, a prospective study in infants found that *nc886* methylation in peripheral blood at birth predicts BMI at the age of 5 [95]. Consistent with the DOHaD hypothesis, hypermethylation at the *DUSP22* promoter (another CoRSIV) shows an association between in utero famine exposure and schizophrenia [128]. Methylation at a CoRSIV located in the promotor of the *PM20D1* gene has been linked with Alzheimer's disease [129]. More studies have found associations between CoRSIV gene methylation and Parkinson's disease [130], autism [131, 132], major depression and suicide [133], rheumatoid arthritis [134], multiple sclerosis [135], and obesity [136]. Methylation in SIV regions near the *OR2L13* promoter and gene body of *CYP2E1* [124] is associated with maternal gestational diabetes mellitus [71]. Hence, despite their under-representation on the Illumina arrays, CoRSIVs are often among top hits in HM450 and EPIC profiling studies screening for associations with disease and associated phenotypes, indicating immense potential for these regions to contribute to disease prediction, diagnosis, and prognosis. From a DOHaD perspective, a focus on CoRSIVs is particularly warranted, given their

well-documented plasticity to periconceptional environment [28, 62, 63, 121, 122, 137].

In addition to a focus on CoRSIVs, we believe the field will benefit from development of novel analytical approaches. Most studies of DNA methylation and disease have utilized univariate regression methodologies and focused on detecting associations rather than making predictions [138, 139]. It is increasingly recognized, however, that individual CpG sites do not provide as much information as coordinated interactions among multiple CpGs. Multivariate approaches can harness crucial synergistic biological effects [140], motivating increased interest in using machine learning to analyze DNA methylation. Target-capture approaches to study DNA methylation across the entire set of known CoRSIVs are under development. Meanwhile, there are many publicly available HM450 and EPIC datasets, in which ~10% of known CoRSIVs are covered by at least one probe. A recent study [141] took advantage of a publicly available HM450 data set on peripheral blood of schizophrenia (SZ) cases and controls [142] to develop a CoRSIV-focused machine learning classifier based on sparse partial least squares discriminant analysis (SPLS-DA). The model calculated an epigenetic risk score which was able to identify SZ cases with 80% positive predictive value, far surpassing the performance of an analogous SPLS-DA classifier based on polygenic risk score. Additional analyses indicated that these associations were not due to reverse causality, as might be caused by the tendency for SZ patients to smoke heavily and/or take psychotropic medications. Together these findings indicate that the systemic interindividual variants distinguishing SZ cases from controls were present prior to diagnosis; prospective studies will be required to confirm this. Nonetheless, this study provides compelling evidence that a focus on SIV, combined with sophisticated machine learning approaches, may ultimately enable blood-based disease risk prediction for a wide range of complex human diseases, with obvious implications for DOHaD.

6.8 Outlook

An epigenetic basis for DOHaD involves two steps: (1) early environmental influences during critical ontogenic periods can induce lasting epigenetic changes, and (2) these individual epigenetic differences must influence risk of disease later in life. There is now extensive evidence supporting the first step. Particularly in the context of human metastable epialleles and CoRSIVs, it is clear that periconceptional environment affects establishment of DNA methylation states that persist for years [62, 120, 122, 124]. The focus now must be on the second step of the pathway, i.e., establishing causal links between individual epigenetic variation and risk of disease. Despite the "failed start" due to the problems with the Illumina platforms, we believe that an increasing focus on CoRSIVs heralds great potential in the field of epigenetic epidemiology. The systemic nature of interindividual epigenetic variation means that CoRSIVs are essentially epigenetic polymorphisms, facilitating the use of DNA samples from blood, saliva, or buccal cells in large-scale epigenetic epidemiologic studies. Development of commercial

platforms focused on CoRSIVs, coupled with the establishment of prospective longitudinal cohorts, will allow epigenetic epidemiologists to probe causal links between early environment, DNA methylation, and disease.

6.9 Conclusions

The concept of DOHaD arose from epidemiologic studies. Developmental plasticity implies that fetal development adapts to transient nutritional and environmental experiences, resulting in lasting changes in chronic disease susceptibility. While our understanding of the underlying mechanisms is rudimentary, alterations in epigenetic regulation are likely contributors. Although CoRSIVs provide a promising avenue for future DOHaD-centered epigenetic studies, we emphasize that epigenetics is only one of several potential mechanisms explaining developmental plasticity. A better understanding of the mechanisms underlying DOHaD should someday make it possible to reduce individual risk of disease by both preventive strategies targeted to early life and corrective approaches designed to normalize malleable cellular and molecular mechanisms set askew by adverse early exposures.

References

1. Rose G (1964) Familial patterns in ischaemic heart disease. Br J Prev Soc Med 18:75–80. https://doi.org/10.1136/jech.18.2.75
2. Forsdahl A (1977) Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med 31:91–95. https://doi.org/10.1136/jech.31.2.91
3. Barker DJ (1995) Fetal origins of coronary heart disease. BMJ 311:171–174. https://doi.org/10.1136/bmj.311.6998.171
4. Barker DJ, Winter PD, Osmond C et al (1989) Weight in infancy and death from ischaemic heart disease. Lancet 2:577–580. https://doi.org/10.1016/s0140-6736(89)90710-1
5. Barker DJ (1997) Intrauterine programming of coronary heart disease and stroke. Acta Paediatr Suppl 423:178–182.; discussion 183. https://doi.org/10.1111/j.1651-2227.1997.tb18408.x
6. Barker DJ, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. BMJ 301:259–262. https://doi.org/10.1136/bmj.301.6746.259
7. Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1:1077–1081. https://doi.org/10.1016/s0140-6736(86)91340-1
8. Hales CN, Barker DJ, Clark PM et al (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022. https://doi.org/10.1136/bmj.303.6809.1019
9. Curhan GC, Chertow GM, Willett WC et al (1996) Birth weight and adult hypertension and obesity in women. Circulation 94:1310–1315. https://doi.org/10.1161/01.cir.94.6.1310
10. Rich-Edwards JW, Stampfer MJ, Manson JE et al (1997) Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. BMJ 315:396–400. https://doi.org/10.1136/bmj.315.7105.396

11. Andersson SW, Lapidus L, Niklasson A et al (2000) Blood pressure and hypertension in middle-aged women in relation to weight and length at birth: a follow-up study. J Hypertens 18:1753–1761. https://doi.org/10.1097/00004872-200018120-00008
12. Ravelli GP, Stein ZA, Susser MW (1976) Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 295:349–353. https://doi.org/10.1056/NEJM197608122950701
13. Ravelli AC, van der Meulen JH, Michels RP et al (1998) Glucose tolerance in adults after prenatal exposure to famine. Lancet 351:173–177. https://doi.org/10.1016/s0140-6736(97)07244-9
14. Susser ES, Lin SP (1992) Schizophrenia after prenatal exposure to the Dutch hunger Winter of 1944-1945. Arch Gen Psychiatry 49:983–988. https://doi.org/10.1001/archpsyc.1992.01820120071010
15. Forsén T, Eriksson JG, Tuomilehto J et al (1997) Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. BMJ 315:837–840. https://doi.org/10.1136/bmj.315.7112.837
16. Sørensen HT, Sabroe S, Rothman KJ et al (1997) Relation between weight and length at birth and body mass index in young adulthood: cohort study. BMJ 315:1137. https://doi.org/10.1136/bmj.315.7116.1137
17. Stuebe AM, Forman MR, Michels KB (2009) Maternal-recalled gestational weight gain, pre-pregnancy body mass index, and obesity in the daughter. Int J Obes 33:743–752. https://doi.org/10.1038/ijo.2009.101
18. Lawlor DA, Lichtenstein P, Långström N (2011) Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. Circulation 123:258–265. https://doi.org/10.1161/CIRCULATIONAHA.110.980169
19. Michels KB, Xue F (2006) Role of birthweight in the etiology of breast cancer. Int J Cancer 119:2007–2025. https://doi.org/10.1002/ijc.22004
20. Michels KB, Trichopoulos D, Robins JM et al (1996) Birthweight as a risk factor for breast cancer. Lancet 348:1542–1546. https://doi.org/10.1016/S0140-6736(96)03102-9
21. Caughey RW, Michels KB (2009) Birth weight and childhood leukemia: a meta-analysis and review of the current evidence. Int J Cancer 124:2658–2670. https://doi.org/10.1002/ijc.24225
22. Harder T, Plagemann A, Harder A (2008) Birth weight and subsequent risk of childhood primary brain tumors: a meta-analysis. Am J Epidemiol 168:366–373. https://doi.org/10.1093/aje/kwn144
23. Michos A, Xue F, Michels KB (2007) Birth weight and the risk of testicular cancer: a meta-analysis. Int J Cancer 121:1123–1131. https://doi.org/10.1002/ijc.22771
24. Hales CN, Barker DJ (2001) The thrifty phenotype hypothesis. Br Med Bull 60:5–20. https://doi.org/10.1093/bmb/60.1.5
25. Hochberg Z, Feil R, Constancia M et al (2011) Child health, developmental plasticity, and epigenetic programming. Endocr Rev 32:159–224. https://doi.org/10.1210/er.2009-0039
26. Rickard IJ, Lummaa V (2007) The predictive adaptive response and metabolic syndrome: challenges for the hypothesis. Trends Endocrinol Metab 18:94–99. https://doi.org/10.1016/j.tem.2007.02.004
27. Gluckman P, Hanson M (2006) Mismatch. Why our world no longer fits our bodies. Oxford University Press, Oxford
28. Waterland RA, Garza C (1999) Potential mechanisms of metabolic imprinting that lead to chronic disease. Am J Clin Nutr 69:179–197. https://doi.org/10.1093/ajcn/69.2.179
29. Waterland RA, Jirtle RL (2004) Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 20:63–68. https://doi.org/10.1016/j.nut.2003.09.011
30. Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33(Suppl):245–254. https://doi.org/10.1038/ng1089

31. Waterland RA, Michels KB (2007) Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr 27:363–388. https://doi.org/10.1146/annurev.nutr.27.061406.093705
32. Ohlsson R, Nyström A, Pfeifer-Ohlsson S et al (1993) IGF2 is parentally imprinted during human embryogenesis and in the Beckwith-Wiedemann syndrome. Nat Genet 4:94–97. https://doi.org/10.1038/ng0593-94
33. Weksberg R, Shen DR, Fei YL et al (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nat Genet 5:143–150. https://doi.org/10.1038/ng1093-143
34. Gicquel C, Rossignol S, Cabrol S et al (2005) Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. Nat Genet 37:1003–1007. https://doi.org/10.1038/ng1629
35. Ogawa O, Eccles MR, Szeto J et al (1993) Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. Nature 362:749–751. https://doi.org/10.1038/362749a0
36. Steenman MJ, Rainier S, Dobry CJ et al (1994) Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumour. Nat Genet 7:433–439. https://doi.org/10.1038/ng0794-433
37. Cui H, Cruz-Correa M, Giardiello FM et al (2003) Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 299:1753–1755. https://doi.org/10.1126/science.1080902
38. Jelinic P, Shaw P (2007) Loss of imprinting and cancer. J Pathol 211:261–268. https://doi.org/10.1002/path.2116
39. Waterland RA (2009) Early environmental effects on epigenetic regulation in humans. Epigenetics 4:523–525. https://doi.org/10.4161/epi.4.8.10155
40. Carpenter BL, Remba TK, Thomas SL et al (2021) Oocyte age and preconceptual alcohol use are highly correlated with epigenetic imprinting of a noncoding RNA (nc886). Proc Natl Acad Sci U S A 118:e2026580118. https://doi.org/10.1073/pnas.2026580118
41. Morgan HD, Santos F, Green K et al (2005) Epigenetic reprogramming in mammals. Hum Mol Genet 14(1):R47–R58. https://doi.org/10.1093/hmg/ddi114
42. Razin A, Riggs AD (1980) DNA methylation and gene function. Science 210:604–610. https://doi.org/10.1126/science.6254144
43. Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. Science 293:1089–1093. https://doi.org/10.1126/science.1063443
44. Rideout WM, Eggan K, Jaenisch R (2001) Nuclear cloning and epigenetic reprogramming of the genome. Science 293:1093–1098. https://doi.org/10.1126/science.1063206
45. Santos F, Dean W (2004) Epigenetic reprogramming during early development in mammals. Reproduction 127:643–651. https://doi.org/10.1530/rep.1.00221
46. Obata Y, Kono T (2002) Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. J Biol Chem 277:5285–5289. https://doi.org/10.1074/jbc.M108586200
47. Lucifero D, Mann MRW, Bartolomei MS, Trasler JM (2004) Gene-specific timing and epigenetic memory in oocyte imprinting. Hum Mol Genet 13:839–849. https://doi.org/10.1093/hmg/ddh104
48. Tomizawa S, Kobayashi H, Watanabe T et al (2011) Dynamic stage-specific changes in imprinted differentially methylated regions during early mammalian development and prevalence of non-CpG methylation in oocytes. Development 138:811–820. https://doi.org/10.1242/dev.061416
49. Allegrucci C, Thurston A, Lucas E, Young L (2005) Epigenetics and the germline. Reproduction 129:137–149. https://doi.org/10.1530/rep.1.00360
50. Lister R, Mukamel EA, Nery JR et al (2013) Global epigenomic reconfiguration during mammalian brain development. Science 341:1237905. https://doi.org/10.1126/science.1237905

51. Yoder JA, Walsh CP, Bestor TH (1997) Cytosine methylation and the ecology of intragenomic parasites. Trends Genet 13:335–340. https://doi.org/10.1016/s0168-9525(97)01181-5
52. Yoder JA, Soman NS, Verdine GL, Bestor TH (1997) DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. J Mol Biol 270:385–395. https://doi.org/10.1006/jmbi.1997.1125
53. Rakyan VK, Blewitt ME, Druker R et al (2002) Metastable epialleles in mammals. Trends Genet 18:348–351. https://doi.org/10.1016/s0168-9525(02)02709-9
54. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21. https://doi.org/10.1101/gad.947102
55. Brandeis M, Frank D, Keshet I et al (1994) Sp1 elements protect a CpG island from de novo methylation. Nature 371:435–438. https://doi.org/10.1038/371435a0
56. Fowden AL, Sibley C, Reik W, Constancia M (2006) Imprinted genes, placental development and fetal growth. Horm Res 65(Suppl 3):50–58. https://doi.org/10.1159/000091506
57. Coan PM, Burton GJ, Ferguson-Smith AC (2005) Imprinted genes in the placenta--a review. Placenta 26(Suppl A):S10–S20. https://doi.org/10.1016/j.placenta.2004.12.009
58. Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300. https://doi.org/10.1128/MCB.23.15.5293-5300.2003
59. Wolff GL, Kodell RL, Moore SR, Cooney CA (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 12:949–957
60. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL (2006) Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114:567–572. https://doi.org/10.1289/ehp.8700
61. Waterland RA, Dolinoy DC, Lin J-R et al (2006) Maternal methyl supplements increase offspring DNA methylation at Axin fused. Genesis 44:401–406. https://doi.org/10.1002/dvg.20230
62. Waterland RA, Kellermayer R, Laritsky E et al (2010) Season of conception in rural Gambia affects DNA methylation at putative human metastable epialleles. PLoS Genet 6:e1001252. https://doi.org/10.1371/journal.pgen.1001252
63. Dominguez-Salas P, Moore SE, Baker MS et al (2014) Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. Nat Commun 5:3746. https://doi.org/10.1038/ncomms4746
64. Felix JF, Cecil C a M (2019) Population DNA methylation studies in the developmental origins of health and disease (DOHaD) framework. J Dev Orig Health Dis 10:306–313. https://doi.org/10.1017/S2040174418000442
65. https://www.niehs.nih.gov/research/atniehs/labs/epi/pi/genetics/pace/index.cfm
66. Joubert BR, Felix JF, Yousefi P et al (2016) DNA methylation in Newborns and maternal smoking in pregnancy: genome-wide consortium meta-analysis. Am J Hum Genet 98:680–696. https://doi.org/10.1016/j.ajhg.2016.02.019
67. Everson TM, Vives-Usano M, Seyve E et al (2021) Placental DNA methylation signatures of maternal smoking during pregnancy and potential impacts on fetal growth. Nat Commun 12:5095. https://doi.org/10.1038/s41467-021-24558-y
68. Sharp GC, Salas LA, Monnereau C et al (2017) Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. Hum Mol Genet 26:4067–4085. https://doi.org/10.1093/hmg/ddx290
69. Sharp GC, Arathimos R, Reese SE et al (2018) Maternal alcohol consumption and offspring DNA methylation: findings from six general population-based birth cohorts. Epigenomics 10:27–42. https://doi.org/10.2217/epi-2017-0095
70. Binder AM, LaRocca J, Lesseur C et al (2015) Epigenome-wide and transcriptome-wide analyses reveal gestational diabetes is associated with alterations in the human leukocyte antigen complex. Clin Epigenetics 7:79. https://doi.org/10.1186/s13148-015-0116-y

71. Howe CG, Cox B, Fore R et al (2020) Maternal gestational diabetes mellitus and Newborn DNA methylation: findings from the pregnancy and childhood epigenetics consortium. Diabetes Care 43:98–105. https://doi.org/10.2337/dc19-0524
72. Leseva MN, Binder AM, Ponsonby A-L et al (2020) Differential gene expression and limited epigenetic dysregulation at the materno-fetal interface in preeclampsia. Hum Mol Genet 29: 335–350. https://doi.org/10.1093/hmg/ddz287
73. Kazmi N, Sharp GC, Reese SE et al (2019) Hypertensive disorders of pregnancy and DNA methylation in Newborns. Hypertension 74:375–383. https://doi.org/10.1161/HYPERTENSIONAHA.119.12634
74. Hoyo C, Murtha AP, Schildkraut JM et al (2011) Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. Epigenetics 6: 928–936. https://doi.org/10.4161/epi.6.7.16263
75. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D et al (2009) Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. PLoS One 4:e7845. https://doi.org/10.1371/journal.pone.0007845
76. Joubert BR, den Dekker HT, Felix JF et al (2016) Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun 7:10577. https://doi.org/10.1038/ncomms10577
77. Heijmans BT, Tobi EW, Stein AD et al (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105:17046–17049. https://doi.org/10.1073/pnas.0806560105
78. Tobi EW, Lumey LH, Talens RP et al (2009) DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 18:4046–4053. https://doi.org/10.1093/hmg/ddp353
79. Michels KB, Harris HR, Barault L (2011) Birthweight, maternal weight trajectories and global DNA methylation of LINE-1 repetitive elements. PLoS One 6:e25254. https://doi.org/10.1371/journal.pone.0025254
80. Engel SM, Joubert BR, Wu MC et al (2014) Neonatal genome-wide methylation patterns in relation to birth weight in the Norwegian mother and child cohort. Am J Epidemiol 179:834–842. https://doi.org/10.1093/aje/kwt433
81. Haworth KE, Farrell WE, Emes RD et al (2014) Methylation of the FGFR2 gene is associated with high birth weight centile in humans. Epigenomics 6:477–491. https://doi.org/10.2217/epi.14.40
82. Simpkin AJ, Suderman M, Gaunt TR et al (2015) Longitudinal analysis of DNA methylation associated with birth weight and gestational age. Hum Mol Genet 24:3752–3763. https://doi.org/10.1093/hmg/ddv119
83. Küpers LK, Monnereau C, Sharp GC et al (2019) Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight. Nat Commun 10:1893. https://doi.org/10.1038/s41467-019-09671-3
84. Einstein F, Thompson RF, Bhagat TD et al (2010) Cytosine methylation dysregulation in neonates following intrauterine growth restriction. PLoS One 5:e8887. https://doi.org/10.1371/journal.pone.0008887
85. Apostolidou S, Abu-Amero S, O'Donoghue K et al (2007) Elevated placental expression of the imprinted PHLDA2 gene is associated with low birth weight. J Mol Med (Berl) 85:379–387. https://doi.org/10.1007/s00109-006-0131-8
86. Diplas AI, Lambertini L, Lee M-J et al (2009) Differential expression of imprinted genes in normal and IUGR human placentas. Epigenetics 4:235–240. https://doi.org/10.4161/epi.9019
87. Guo L, Choufani S, Ferreira J et al (2008) Altered gene expression and methylation of the human chromosome 11 imprinted region in small for gestational age (SGA) placentae. Dev Biol 320:79–91. https://doi.org/10.1016/j.ydbio.2008.04.025
88. McMinn J, Wei M, Schupf N et al (2006) Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 27:540–549. https://doi.org/10.1016/j.placenta.2005.07.004

89. Chelbi ST, Doridot L, Mondon F et al (2011) Combination of promoter hypomethylation and PDX1 overexpression leads to TBX15 decrease in vascular IUGR placentas. Epigenetics 6: 247–255. https://doi.org/10.4161/epi.6.2.13791
90. Fryer AA, Emes RD, Ismail KMK et al (2011) Quantitative, high-resolution epigenetic profiling of CpG loci identifies associations with cord blood plasma homocysteine and birth weight in humans. Epigenetics 6:86–94. https://doi.org/10.4161/epi.6.1.13392
91. Ferreira JC, Choufani S, Grafodatskaya D et al (2011) WNT2 promoter methylation in human placenta is associated with low birthweight percentile in the neonate. Epigenetics 6:440–449. https://doi.org/10.4161/epi.6.4.14554
92. Filiberto AC, Maccani MA, Koestler D et al (2011) Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 6:566–572. https://doi.org/10.4161/epi.6.5.15236
93. Godfrey KM, Sheppard A, Gluckman PD et al (2011) Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes 60:1528–1534. https://doi.org/10.2337/db10-0979
94. Lillycrop K, Murray R, Cheong C et al (2017) ANRIL promoter DNA methylation: a perinatal marker for later adiposity. EBioMedicine 19:60–72. https://doi.org/10.1016/j.ebiom.2017.03.037
95. van Dijk SJ, Peters TJ, Buckley M et al (2018) DNA methylation in blood from neonatal screening cards and the association with BMI and insulin sensitivity in early childhood. Int J Obes 42:28–35. https://doi.org/10.1038/ijo.2017.228
96. Cardenas A, Rifas-Shiman SL, Agha G et al (2017) Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Sci Rep 7:288. https://doi.org/10.1038/s41598-017-00384-5
97. Bianco-Miotto T, Craig JM, Gasser YP et al (2017) Epigenetics and DOHaD: from basics to birth and beyond. J Dev Orig Health Dis 8:513–519. https://doi.org/10.1017/S2040174417000733
98. Cortese R, Lu L, Yu Y et al (2016) Epigenome-microbiome crosstalk: a potential new paradigm influencing neonatal susceptibility to disease. Epigenetics 11:205–215. https://doi.org/10.1080/15592294.2016.1155011
99. Yu D-H, Gadkari M, Zhou Q et al (2015) Postnatal epigenetic regulation of intestinal stem cells requires DNA methylation and is guided by the microbiome. Genome Biol 16:211. https://doi.org/10.1186/s13059-015-0763-5
100. Kumar H, Lund R, Laiho A et al (2014) Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. MBio 5:e02113–e02114. https://doi.org/10.1128/mBio.02113-14
101. Paschos K, Allday MJ (2010) Epigenetic reprogramming of host genes in viral and microbial pathogenesis. Trends Microbiol 18:439–447. https://doi.org/10.1016/j.tim.2010.07.003
102. Mischke M, Plösch T (2016) The gut microbiota and their metabolites: potential implications for the host epigenome. Adv Exp Med Biol 902:33–44. https://doi.org/10.1007/978-3-319-31248-4_3
103. Remely M, Aumueller E, Merold C et al (2014) Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. Gene 537:85–92. https://doi.org/10.1016/j.gene.2013.11.081
104. International HapMap Consortium (2003) The international HapMap project. Nature 426:789–796. https://doi.org/10.1038/nature02168
105. Feinberg AP, Irizarry RA (2010) Evolution in health and medicine Sackler colloquium: stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. Proc Natl Acad Sci U S A 107(Suppl 1):1757–1764. https://doi.org/10.1073/pnas.0906183107
106. Petronis A (2010) Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature 465:721–727. https://doi.org/10.1038/nature09230

107. Kulis M, Esteller M (2010) DNA methylation and cancer. Adv Genet 70:27–56. https://doi.org/10.1016/B978-0-12-380866-0.60002-2
108. Kulis M, Queirós AC, Beekman R, Martín-Subero JI (2013) Intragenic DNA methylation in transcriptional regulation, normal differentiation and cancer. Biochim Biophys Acta 1829: 1161–1174. https://doi.org/10.1016/j.bbagrm.2013.08.001
109. Rakyan VK, Down TA, Balding DJ, Beck S (2011) Epigenome-wide association studies for common human diseases. Nat Rev Genet 12:529–541. https://doi.org/10.1038/nrg3000
110. Bibikova M, Barnes B, Tsan C et al (2011) High density DNA methylation array with single CpG site resolution. Genomics 98:288–295. https://doi.org/10.1016/j.ygeno.2011.07.007
111. Pidsley R, Zotenko E, Peters TJ et al (2016) Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol 17:208. https://doi.org/10.1186/s13059-016-1066-1
112. Grundberg E, Meduri E, Sandling JK et al (2013) Global analysis of DNA methylation variation in adipose tissue from twins reveals links to disease-associated variants in distal regulatory elements. Am J Hum Genet 93:876–890. https://doi.org/10.1016/j.ajhg.2013.10.004
113. Zaimi I, Pei D, Koestler DC et al (2018) Variation in DNA methylation of human blood over a 1-year period using the Illumina MethylationEPIC array. Epigenetics 13:1056–1071. https://doi.org/10.1080/15592294.2018.1530008
114. Bose M, Wu C, Pankow JS et al (2014) Evaluation of microarray-based DNA methylation measurement using technical replicates: the atherosclerosis risk in communities (ARIC) study. BMC Bioinformatics 15:312. https://doi.org/10.1186/1471-2105-15-312
115. Hachiya T, Furukawa R, Shiwa Y et al (2017) Genome-wide identification of inter-individually variable DNA methylation sites improves the efficacy of epigenetic association studies. NPJ Genom Med 2:11. https://doi.org/10.1038/s41525-017-0016-5
116. Gallego-Paüls M, Hernández-Ferrer C, Bustamante M et al (2021) Variability of multi-omics profiles in a population-based child cohort. BMC Med 19:166. https://doi.org/10.1186/s12916-021-02027-z
117. Logue MW, Smith AK, Wolf EJ et al (2017) The correlation of methylation levels measured using Illumina 450K and EPIC BeadChips in blood samples. Epigenomics 9:1363–1371. https://doi.org/10.2217/epi-2017-0078
118. Wahl S, Drong A, Lehne B et al (2017) Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 541:81–86. https://doi.org/10.1038/nature20784
119. Viana J, Hannon E, Dempster E et al (2017) Schizophrenia-associated methylomic variation: molecular signatures of disease and polygenic risk burden across multiple brain regions. Hum Mol Genet 26:210–225. https://doi.org/10.1093/hmg/ddw373
120. Silver MJ, Kessler NJ, Hennig BJ et al (2015) Independent genomewide screens identify the tumor suppressor VTRNA2-1 as a human epiallele responsive to periconceptional environment. Genome Biol 16:118. https://doi.org/10.1186/s13059-015-0660-y
121. Kessler NJ, Waterland RA, Prentice AM, Silver MJ (2018) Establishment of environmentally sensitive DNA methylation states in the very early human embryo. Sci Adv 4:eaat2624. https://doi.org/10.1126/sciadv.aat2624
122. Gunasekara CJ, Scott CA, Laritsky E et al (2019) A genomic atlas of systemic interindividual epigenetic variation in humans. Genome Biol 20:105. https://doi.org/10.1186/s13059-019-1708-1
123. GTEx Consortium (2013) The genotype-tissue expression (GTEx) project. Nat Genet 45:580–585. https://doi.org/10.1038/ng.2653
124. Van Baak TE, Coarfa C, Dugué P-A et al (2018) Epigenetic supersimilarity of monozygotic twin pairs. Genome Biol 19:2. https://doi.org/10.1186/s13059-017-1374-0
125. Dugué P-A, Yu C, McKay T et al (2021) VTRNA2-1: genetic variation, heritable methylation and disease association. Int J Mol Sci 22:2535. https://doi.org/10.3390/ijms22052535

126. Lin C-H, Lee Y-S, Huang Y-Y, Tsai C-N (2021) Methylation status of vault RNA 2-1 promoter is a predictor of glycemic response to glucagon-like peptide-1 analog therapy in type 2 diabetes mellitus. BMJ Open Diabetes Res Care 9:e001416. https://doi.org/10.1136/bmjdrc-2020-001416
127. You Y-A, Kwon EJ, Hwang H-S et al (2021) Elevated methylation of the vault RNA2-1 promoter in maternal blood is associated with preterm birth. BMC Genomics 22:528. https://doi.org/10.1186/s12864-021-07865-y
128. Boks MP, Houtepen LC, Xu Z et al (2018) Genetic vulnerability to DUSP22 promoter hypermethylation is involved in the relation between in utero famine exposure and schizophrenia. NPJ Schizophr 4:16. https://doi.org/10.1038/s41537-018-0058-4
129. Sanchez-Mut JV, Heyn H, Silva BA et al (2018) PM20D1 is a quantitative trait locus associated with Alzheimer's disease. Nat Med 24:598–603. https://doi.org/10.1038/s41591-018-0013-y
130. Young JI, Sivasankaran SK, Wang L et al (2019) Genome-wide brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease. Neurol Genet 5:e342. https://doi.org/10.1212/NXG.0000000000000342
131. Dall' Aglio L, Muka T, Cecil CAM et al (2018) The role of epigenetic modifications in neurodevelopmental disorders: a systematic review. Neurosci Biobehav Rev 94:17–30. https://doi.org/10.1016/j.neubiorev.2018.07.011
132. Zhao B-H, Jiang Y, Zhu H et al (2019) Placental Delta-like 1 gene DNA methylation levels are related to mothers' blood glucose concentration. J Diabetes Res 2019:9521510. https://doi.org/10.1155/2019/9521510
133. Murphy TM, Crawford B, Dempster EL et al (2017) Methylomic profiling of cortex samples from completed suicide cases implicates a role for PSORS1C3 in major depression and suicide. Transl Psychiatry 7:e989. https://doi.org/10.1038/tp.2016.249
134. Mok A, Rhead B, Holingue C et al (2018) Hypomethylation of CYP2E1 and DUSP22 promoters associated with disease activity and erosive disease among rheumatoid arthritis patients. Arthritis Rheumatol 70:528–536. https://doi.org/10.1002/art.40408
135. Maltby VE, Lea RA, Sanders KA et al (2017) Differential methylation at MHC in CD4+ T cells is associated with multiple sclerosis independently of HLA-DRB1. Clin Epigenetics 9:71. https://doi.org/10.1186/s13148-017-0371-1
136. Kühnen P, Handke D, Waterland RA et al (2016) Interindividual variation in DNA methylation at a putative POMC metastable Epiallele is associated with obesity. Cell Metab 24:502–509. https://doi.org/10.1016/j.cmet.2016.08.001
137. Estill MS, Bolnick JM, Waterland RA et al (2016) Assisted reproductive technology alters deoxyribonucleic acid methylation profiles in bloodspots of newborn infants. Fertil Steril 106:629–639.e10. https://doi.org/10.1016/j.fertnstert.2016.05.006
138. Bzdok D, Varoquaux G, Steyerberg EW (2021) Prediction, not association, paves the road to precision medicine. JAMA Psychiatry 78:127–128. https://doi.org/10.1001/jamapsychiatry.2020.2549
139. Rutherford S (2020) The promise of machine learning for psychiatry. Biol Psychiatry 88:e53–e55. https://doi.org/10.1016/j.biopsych.2020.08.024
140. Wiemken TL, Kelley RR (2020) Machine learning in epidemiology and health outcomes research. Annu Rev Public Health 41:21–36. https://doi.org/10.1146/annurev-publhealth-040119-094437
141. Gunasekara CJ, Hannon E, MacKay H et al (2021) A machine learning case-control classifier for schizophrenia based on DNA methylation in blood. Transl Psychiatry 11:412. https://doi.org/10.1038/s41398-021-01496-3
142. Hannon E, Dempster E, Viana J et al (2016) An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential DNA methylation. Genome Biol 17:176. https://doi.org/10.1186/s13059-016-1041-x