Nucleus (2021) 64:259–270
https://doi.org/10.1007/s13237-021-00367-y

REVIEW ARTICLE

DNA methylation and regulation of gene expression: Guardian of our health

Gaurab Aditya Dhar[1] · Shagnik Saha[1] · Parama Mitra[1] · Ronita Nag Chaudhuri[1]

Received: 18 May 2021 / Accepted: 30 July 2021 / Published online: 16 August 2021

Abstract
One of the most critical epigenetic signatures present in the genome of higher eukaryotes is the methylation of DNA at the C-5 position of the cytosine ring. Based on the sites of DNA methylation in a locus, it can serve as a repressive or activation mark for gene expression. In a crosstalk with histone modifiers, DNA methylation can consequently either inhibit binding of the transcription machinery or generate a landscape conducive for transcription. During developmental phases, the DNA methylation pattern in the genome undergoes alterations as a result of regulated balance between de novo DNA methylation and demethylation. Resultantly, differentiated cells inherit a unique DNA methylation pattern that fine tunes tissue-specific gene expression. Although apparently a stable epigenetic mark, DNA methylation is actually labile and is a complex reflection of interaction between epigenome, genome and environmental factors prior to birth and during progression of life. Recent findings indicate that levels of DNA methylation in an individual is a dynamic outcome, strongly influenced by the dietary environment during germ cell formation, embryogenesis and post birth exposures. Loss of balances in DNA methylation during developmental stages may result in imprinting disorders, while at any later stage may lead to increased predisposition to various diseases and abnormalities. This review aims to provide an outline of how our epigenome is uniquely guided by our lifetime of experiences beginning in the womb and how understanding it better holds future possibilities of improvised clinical applications.

Keywords DNA methylation · Nutrition · Genomic imprinting · Gene expression

Introduction

The methyl group is a fairly simple moiety in general but when present at certain pressure points in the genetic template of an organism, wields the power to silence the expression of a gene, sometimes indefinitely. Removal of the methylation mark allows the gene to be cleared for the processes of transcription and translation, finally alleviating the restrictions imposed on its expression. This regulatory stamping of the genome is maintained by a family of enzymes termed as the DNA methylases, or DNMTs. There are three major DNMTs—DNMT1 and DNMT3 with two major isoforms, DNMT3a and DNMT3b [6, 33, 67, 80, 91]. The DNMTs transfer a methyl group from the methyl donor S-adenosylmethionine (SAM) to the cytosine residue in a dinucleotide CG or polynucleotide CGGCGG context, also referred to as CpG islands. The de novo methylases DNMT3a and DNMT3b possess the ability to methylate non-methylated DNA while the maintenance methylase DNMT1 methylates the non-methylated strand of a hemi-methylated DNA molecule, thus maintaining the integrity of the DNA methylome and conferring the nature of self-perpetuation on the methylation mark across generations.

DNA methylation levels in a locus regulates gene expression intricately. Generally, DNA methylation is known to be a repressive mark present predominantly on CpG dinucleotides in somatic cells. Their presence prevents transcriptional activation of genic regions which are meant to be silenced in a cell-type specific manner by impeding recruitment of the transcription machinery. DNA methylation, however, is

Corresponding Editor: Somnath Paul; Reviewers: Dhurjhoti Saha, Tabish Hussain.

Gaurab Aditya Dhar, Shagnik Saha, Parama Mitra these authors have contributed equally to this work.

✉ Ronita Nag Chaudhuri
ronita.nc@sxccal.edu; ronita_7@yahoo.co.in

[1] Department of Biotechnology, St. Xavier's College, 30 Mother Teresa Sarani, Kolkata 700016, India

refractory to certain CpG dense regions which are present at gene promoters called CpG islands (CGIs). The absence of DNA methylation at CGIs is evolutionarily conserved and intricately linked to their property of acting as start sites for transcription initiation [57]. The presence of non-methylated GC rich DNA and/or CGIs at promoters allows DNA to adapt a conformation that destabilises nucleosomes and facilitates the binding of required factors for initiation of transcription. This also allows a cross-talk between different post translational modifications (PTMs) on histone residues to promote a chromatin environment favourable for transcription. DNA methylation, especially when present within intronic regions, often recruit specific histone modifiers and chromatin remodellers that cause increased transcription from the locus. Thus, whether DNA methylation would suppress or activate gene expression is context dependent and the actual effect of $5^{m}C$ marks on the DNA strands is very much locus and stimulus dependent. The prenatal environment and diet can strongly influence the methylation status of foetal DNA, the effect of which is sustained lifelong after birth. Lifestyle adaptations post birth and during adulthood can also affect chromatin regulation and gene expression through DNA methylation and associated histone modifications, thus affecting health and overall well-being of an individual. Here we review recent findings and provide a comprehensive picture on the influence of perinatal and adulthood exposure to various factors that leads to altered DNA methylation, gene expression and a predisposition to various diseases.

DNA methylation and Genomic Imprinting

With a progressive development on the concept of genomic imprinting over the past decades, significance of DNA methylation and genomic fidelity has gained heights. The $5^{m}C$ marks at specific CpG Islands are in a dynamic balance across generations—an event of erasure in the gametes, followed by imprinting of the alleles based on their parental origin by the different DNA methylases in the next generation [51]. It is to be noted that for most of the genes two working copies are inherited one from each parent. However, for imprinted genes only 1 working copy is inherited. Depending on the gene either the copy from mother or from father is epigenetically silenced, through addition of methyl groups during egg or sperm formation. Epigenetic tags added to the genes usually remain intact for the rest of the life and gets reset only during egg or sperm formation. Usually, soon after fertilization, epigenetic tags of gene silencing or activation are removed from DNA. However, in mammals, imprinted genes retain their tags, i.e., these genes begin their functions with earlier epigenetic tags. Interestingly in mammals, the maternally and paternally inherited genomes undergo global demethylation post-fertilization, with differential methylation occurring between maternal and paternal IC alleles before and after implantation. Transposable elements, promoter regions of housekeeping genes and cis-acting regulatory elements of imprinted genes are three key epigenetic susceptibility targets containing CpG sites that need to be methylated, unmethylated and differentially methylated, respectively during development.

An influence of the maternal diet on the foetal gene expression pattern was elucidated as early as 1998 by Wolff and his colleagues, where agouti gene expression in A^{vy}/a mice in the F_1 generation was affected by methyl supplements in the diet of F_0 maternal mice [15, 83]. Research that followed thereafter, have identified the molecular players involved in regulating the DNA methylation pattern of different loci. Progress in sequencing strategies and other sophisticated methods for characterizing the epigenome gave an impetus to this field of research. Recently, it was shown by Yang et al., that maternal diet supplemented with betaine (trimethyl glycine), a potential methyl donor in the biological system, leads to hypermethylation of the Imprinting Control Region of the *Igf2/H19* locus leading to upregulation of *IGF2* gene expression [89]. It has been further reported that betaine supplementation can even influence the F_2 generation progenies [88]. Interestingly, when grandams are supplemented with betaine in their diet, the foetus (F_1 generation) and its germ cells destined to become F_2 offspring both bore the effect of betaine exposure [88, 89]. The F_1 offspring may be directly exposed to betaine transmission via placenta and mother's milk, while the F_2 generation through possible epigenetic modifications on the F_1 gametes [88, 89]. *Igf2/H19* ICR methylation levels and consequent gene expression regulations have been correlated with cerebellar weight [68] and subcutaneous adiposity [38]. Furthermore, there has been evidence that maternal diet influences the methylation pattern of the CpG rich promoters of Retinoid X Receptor Alpha (RXRα) gene, thereby regulating metabolism levels in the offspring [9]. Interesting correlations have been established between the methylation levels at the loci of oxytocin receptor (OXTR) and social and emotional behaviour of humans [64].

Notably, imprinting is unique to mammals and flowering plants. In mammals about 1% of the total gene population are imprinted. As discussed above it is evident how environmental factors and diet that affect DNA methylation patterning during development can potentially influence adult phenotype via alterations in CpG methylation at epigenetically labile regions of the genome. It is therefore pertinent to conclude that influence of parental lifestyle on the offspring health can have manifold effects through regulation of the DNA methylome.

Maternal dietary and supplementary intakes

Role of Vitamins

The fact that health and dietary habits of mothers influences the well-being of offspring(s) is a well-known concept. However, the link between such age-old concept and the epigenetic basis was not so clearly understood until recently. It is a common practice to prescribe various vitamin and mineral supplements to expecting mothers to prevent various offspring disorders like neural tube defect (NTD). A recent study reported that an optimum ratio of vitamin B9 (folic acid) and vitamin B12 (cyanocobalamin) is required to ensure proper development of the offspring. The authors have shown that either over-supplemented or under-supplemented intake of folate and vitamin B12 in prenatal diets can affect global methylation levels of the foetal DNA as well as the functioning of some regulatory mi-RNAs [61]. Significant changes in the ratio of vitamin intake were found to affect the CpG island methylation pattern in the loci of DNMT1, DNMT3A and DNMT3B, consequently leading to increased expression of the methyltransferases [29]. Even levels of miRNAs like that of miR-133 (linked to muscle and cardiac tissue metabolism and certain kinds of cancer) [54, 58, 65, 85, 86, 98, 101] and miR-221 (linked to oncogenesis) [10, 25, 50, 56, 60, 71, 79, 90, 96, 100] changed in response to a skewed ratio of intake of the two vitamins. Other than sub or supra-optimal levels of folate or B12 intake, vitamin C or ascorbic acid consumption during the perinatal period also seems significant [19]. A progressive demethylation during the foetal development by the demethylase TET1 is essential to allow expression of several genes responsible for the development of foetal gonads, especially in the ovaries [19]. Ascorbic acid is an essential cofactor for the TET group of enzymes and recent research shows that female progenies of vitamin C-deficient mice have significantly reduced number of primordial germ cells compared to control mice [19]. The effect of Vitamin C was particularly significant during early embryonic stage, especially before embryonic day 13.5 (the point of lowest genomic methylation levels during germline reprogramming [19]). Foetal testes are not influenced much by ascorbic acid since the meiotic procedures in male foetus are not influenced in utero. But are other pathways influenced by vitamin intake other than through mediation by DNMTs or TETs? Interestingly, evidence of influence of supplements on one carbon metabolism and DNA methylation levels have been elucidated [14, 15, 52, 83]. However, how dietary intake of such supplements during the perinatal period affect epigenetic regulation and consequently metabolism is still elusive. Further experiments are needed to get a more comprehensive view of the gene regulatory landscape during foetal development in response to maternal diet.

Effect on Fat metabolism

Fat metabolism and obesity related processes can also be influenced by maternal nutrition through an effect on the DNA methylome. To gain insight into the contribution of maternal diet on foetal metabolic programming through epigenetic changes in utero, Daniels et al. studied maternal diet and placenta leptin methylation in a group of mother-infant dyads in the immediate postpartum period [16]. Leptin is known for its role to control the food and energy homeostasis in the body via impacting the nervous system, adipocytes and energy reserves in the body. From statistical sampling, the study predicted that greater amounts of carbohydrate intake led to reduction in placenta leptin methylation [16]. Another study in expecting mothers showed that the glycemic index (GI) of maternal diet can influence the methylome of the placental tissue [87]. Genome wide differential DNA methylation analysis done on tissue samples revealed that genes like Perilipin1 (*PLIN1*), involved in fat mobilization and Somatostatin Receptor Type 4 (*SSTR4*), involved in somatostatin signalling to regulate metabolism, had CpGIs whose methylation levels were found to correlate with maternal dietary glycemic index changes [87]. These genes have been implicated in diabetes, maternal obesity, insulin resistance and foetal metabolome regulation [87]. However, in most cases the modality of such experiments was based primarily on human models with sample collection, processing, bioinformatic and statistical analyses. Precise in vivo analyses are further required to elucidate the key molecular players involved in regulating gene expression in foetus exposed to different dietary and supplementary intake of the mothers. Recently, maternal high fat diet has been shown to increase the risk of obesity and diabetes in the offspring [44, 92]. Progeny of mother mice treated to high fat diet showed an increase in body weight and a tendency towards obesity [44]. Lactation at the post-birth phase exposes the offspring to milk lipid components that helps to activate fatty acid metabolism specific genes like *FGF21* through a progressive event of demethylation [92].

Intriguingly, it is thus evident that not only dietary exposure in utero, but malnutrition in early life has the ability to affect health and well-being of an adult. Work of Yuan et al., mentioned above, brings into light how obesity may not be just a lifestyle problem, but may be traced back to the post-birth dietary intake of an individual. However, to gain further insight into the field and designate precise epigenetic players involved at specific loci further experimentation in mouse models are required. Data generated from such research would clearly validate the significance of DNA

methylation and gene expression in a developing foetus and how it is influenced by maternal nutritional make up.

Environmental factors and epigenetic regulations "in utero"

Environmental pollutants can also have serious consequences on the foetal development in utero by affecting the epigenome. Studies indicate interaction between potentially toxic metals and essential elements with the global and gene-specific methylation of DNA repair (8-oxoguanine DNA glycosylase, *OGG1* and poly (ADP-ribose) polymerase 1, *PARP1*) and antioxidant (nuclear factor erythroid 2-related factor 2, *NRF2*) genes. Such correlations were reported in new-borns prenatally exposed to environmental metals [66]. Arsenic and mercury exposure increased methylation levels of Long Interspersed Repetitive Element 1 (LINE1) at low zinc concentrations, while an increased zinc concentration reduced the methylation levels and rescued the progeny from arsenic and mercury induced hypermethylation [66]. It is well established that an alteration in the expression of antioxidant genes and DNA damage repair enzymes can cause predisposition to cancer and chromosomal anomalies. Intriguingly, exposure to diesel exhaust during the prenatal period modified gene expression levels of around 300 genes in the F_1 generation progenies and many of these genes were found to be involved in cardiac metabolism [32]. It was observed that, in utero exposure to diesel exhaust and the consequent alteration in gene expression mimics changes observed in hypertrophic neonatal cardiomyocytes. Some of these genes were identified to be involved in mitochondrial L-carnitine shuttle pathway, fatty acid beta-oxidation and AMPK signalling. Further studies revealed 62 differentially methylated regions (DMRs) on the DNA, and these were identified to be in intronic and intergenic regions of metabolism specific genes like *GNAS*, *GNG12* and *PDE6H* [32]. Metabolic dysregulation is known to be a hallmark feature of heart failure. Moreover, passive removal of DNA methylation can also occur due to excessive oxidative damage from reactive oxygen species to such an extent that DNA methyltransferases (DNMTs) are unable to bind to their specific substrate, thereby changing the methylation status in daughter cells [32]. If such an event occurs early in development during the repatterning of methylation, these changes would be heritable and is likely to occur in multiple cell and organ types.

Effect of diet during adult life

For adults, regular diet and specific nutritional intake has profound influences on epigenetic landscape and chromatin regulation of gene expression. Folate and Vitamin B12, for example, are necessary for the synthesis of Methionine and SAM, which are crucial in the maintenance of DNA methylation patterns in specific loci. Inadequate folate intake has long been linked to increased risk of colorectal cancer [30]. Colorectal cancer, in fact is known to be a manifestation of altered epigenetics and resultant genetic changes in epithelial cells of the colon during neoplastic transformation. Altered DNA methylation in specific gene promoters have been designated as the primary cause leading to tumorigenesis in humans (Reviewed in [45]). This has been discussed in several reviews and will presently not be discussed in the present review. But what is interesting to note is that present research is directed towards identifying aberrant DNA methylation patterns. Detection of such CpG island methylator phenotype can be clinically used for early diagnosis as well as prognosis of colorectal cancer (Reviewed in [95]). Reduced DNA methylation in lymphocytes of healthy postmenopausal women have also been linked to folate deficiency [41]. Furthermore, a recent study involving deep bisulfite sequencing to assess DNA methylation pattern has indicated that altered methylation levels at CpG islands can be correlated to hereditary as well as early onset of breast and ovarian cancer [12].

Plant metabolites like polyphenols have been found to cause alterations in the activities of DNA methyltransferases. Hypermethylation in the genes like CDX2 (codes for a transcription factor involved in the development and functioning of the intestinal epithelial cells) and BMP2 (acts as tumor suppressor in gastric carcinoma) have been found in patients with primary gastrocarcinoma. Such individuals have been linked to a history of low intake of cruciferous vegetables and green tea [93, 94]. EGCG, a polyphenol abundantly found in green tea compared to more commonly consumed black tea, may act as an inhibitor of methyltransferases. Such impairment of methyltransferase function is shown to induce expression of genes, originally silenced by methylation in cancer cell lines [24]. *Grapes are not sour at all!* Two phytochemicals Dihydrocafeic acid (DHCA) and malvidin-3'-O-glucoside found in grape juice and grape seed extracts attenuate depression like behaviour, by reducing expression of *DNMT1* that methylates promoters of *Interleukin6* (*IL-6*) genes- thus reducing level of pro-inflammatory cytokine responsible for depressive disorders. DHCA can reportedly decrease the methylation of CpG islands in introns 1 and 3 of the *IL-6* gene, which serve as enhancers, thus reducing its expression (81). Dietary intake of fish oils and omega-3 polyunsaturated fatty acids are known to decrease the DNA methylation of leucocyte *ABCA1* gene, which is a key player in the regulation of HDL-C concentration of blood and hence can be associated with cardiovascular health [27]. Minerals like Selenium in diet can reactivate hypermethylated genes like *GSTP1, APC* and *CSR1* by downregulating the expression of DNMTs in prostate cancer cells [43, 84].

It can be thus summarized that the diet of an individual starting from the "in utero" stage to adulthood holds a huge significance in terms of regular health and disease prevention, through modulation of DNA methylation and consequently the epigenome. Introduction of a new perspective termed "Epigenetic diet" presently focus on consuming products including fruits, vegetables and dietary components that show the ability to stimulate beneficial epigenetic modifications. Such a dietary balance effectively helps to regulate the functionality and fidelity of our genes better.

Effect of present-day lifestyle adaptations

The urban lifestyle of the twentieth century has several drawbacks which seem to have dire effects on human health. In this competitive era, the transition from adolescence to adulthood is often characterized by drastic psychological and physical changes in an individual. In course of the required adaptations, adults often get exposed to psychological stress and an irregular lifestyle. Such adaptations might lead to the development of psychiatric disorders like Major Depressive Disorder (MDD). According to the data report by World Health Organization, over 264 million people suffer from depression globally and 80,000 people die every year of suicide. Intriguingly, interaction between the environment and consequent gene expression is mediated by epigenetic regulations. DNA methylation, in particular, has been reported to play a very significant role in the development of MDD [39]. A number of candidate genes have been found to have differential DNA methylation levels at their promoters in a depressed individual compared to that of a healthy individual. For example, Brain Derived Neurotrophic Factor (Bdnf), which plays an important role in the neurotransmission, synaptic plasticity and regulation of receptor sensitivity in mature neurons, has reduced expression during depression. Such decreased expression of Bdnf has been correlated to increased DNA methylation in its promoter region [26]. Increased methylation at CpG islands in the promoters of *SLC6A4* gene (a serotonin transporter, integral to serotonin signalling in the brain) and that of *NR3C1* (codes for a glucocorticoid receptor) have been associated with depression [11]. Corticotropin releasing factor (CRF) in the paraventricular nucleus (PVN) of the hypothalamus is increased in mice susceptible to chronic social defeat stress, accompanied by decreased DNA methylation of the *Crf* promoter [22]. Additionally, a common practice- drinking water from plastic water bottles has been proven to be harmful due to Bisphenol A (BPA) which is a potent endocrine disruptor [5, 53]. More harmful effects of BPA have been elucidated in BALB/c mice where in utero exposure to BPA induces a prominent change in DNA methylation of the Brain Derived Neurotrophic Factor (*Bdnf*) gene in the hippocampus and blood of F_1 progeny [49]. Studies indicate that DNA methylation status of *BDNF* may be an important marker for the early detection of neurodevelopmental abnormalities [49]. Their findings significantly suggest that high maternal BPA exposure during pregnancy is associated with altered *Bdnf* methylation in the cord blood. Whether the effect of BDNF deficiency will result in a specific psychiatric disorder likely depends on a complex interaction of the genetic make-up, gender and life-long environmental exposure of an individual [49].

Adults whose lifestyle involve unregulated alcohol and tobacco consumption, cigarette smoking and substance abuse, often end up having lasting effects on DNA methylation status of several genes, which eventually might lead to disease development. Alcohol consumption during gestation leads to foetal alcohol spectrum disorders (FASD). Prenatal alcohol exposure (PAE) shows the presence of 118 differentially methylated regions (DMRs) across the hypothalamic tissue samples in rats. Of these, significant methylation level changes were observed in *Ddr4* (Dopaminergic receptor d4) which affects dopamine signalling and implies a potential role in neuropsychiatric disorders in the offspring [59]. Further studies have shown that consumption of alcohol and tobacco alters the DNA methylation patterns of two highly studied genes of the HPA axis, *FKB5* and *N3RC1* [20]. A recent study has shown that, putting an end to smoking habit caused differentially methylated CpG sites in smokers to alter back the methylation level of same CpG sites to a state similar to that of never-smokers, when analysed after 5 years of smoking cessation [42]. Alcohol intake also has its influence on DNA methylation patterns in adults. Glial derived Neurotrophic Factor (Gdnf) is a factor involved in prolonged survival and differentiation of a specific class of neurons. *GDNF* gene expression is alcohol-responsive and is shown to be upregulated in response to short-term alcohol intake and downregulated with the withdrawal of this habit [4]. In consonance, methylation studies showed that withdrawal cases reduced methylation compared to controls, in the Negative Regulatory Elements (NRE) present in exon 1 of *GDNF* locus. Methylation status of NRE act as a regulator of *GDNF* expression (78). While GDNF expression in specific regions of the brain regulates alcohol intake and suppresses alcohol consumption, excess alcohol intake dysregulates such inherent regulatory mechanism.

The COVID-19 pandemic has hit the world hard starting from the year 2020 and has caused massive loss of lives and damage in terms of physical and mental health, economic conditions and disrupted our day-to-day activities. Adjusting to the new lifestyle normals have led to disruptions in the biological clock consequently disrupting DNA methylation patterns in several loci leading to aggravation or onset of several disorders. For example, it has been reported that, certain genes (including several imprinted genes), which had been previously shown to be differentially methylated in the

adipose tissues of obese and Type 2 Diabetes patients- compared to healthy individuals, had altered methylation profiles after one night of sleep loss [8]. Loss of sleep reportedly led to increase in methylation of DMRs in the promoter proximal region, near the TSS of specific genes associated with obesity such as *TNXB, TRIM2 and FOXP2* [8]. Altered DNA methylation due to sleep loss was also found near the TSS of the following genes: *CD36, AKR1CL1, HOXA2*, which are involved in adipogenesis [8]. Decrease in methylation was observed in 56 DMRs near the TSS of several imprinted genes like *TSPAN32* (involved in malignancies, hematopoietic cell function, *GNAS* (codes for the alpha subunit of the G protein and thus plays a key role in the classical signal transduction pathway), *INS* (codes for the peptide hormone insulin), and *GFI1* (codes for a transcriptional repressor, crucial in diverse processes as hematopoiesis, oncogenesis) [8]. Lifestyle habits and disruptions thus seem to have significant effects on DNA methylation pattern of diverse plethora of genes and may lead to serious consequences.

Neurological perspective

The link between DNA methylation and memory formation and maintenance has been strongly established. Yet the question that was baffling scientists was how the methylation mark was erased during memory formation and modification of existing memory. The general notion few decades back was that DNA cytosine methylation once established, was irreversible. Yet, for neuronal plasticity and modification of memory, the requirement of dynamicity was as paramount as the need for self-perpetuation. In context of the brain, this was an absolute enigma as the neurons were quiescent and the idea of changes in the methylation code was unthinkable. All of this changed with the discovery that a family of enzymes called the Ten-eleven translocation or TET proteins which erased the methylation marks by oxidizing the 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by an Fe (II) / α-ketoglutarate dependent mechanism, which subsequently undergoes deamination to 5-hydroxy-methyluracil and is repaired via base or nucleotide excision repair machinery [34, 40, 77]. This made the methylome dynamic and potentially plastic and thus, uniquely suitable for analysis concerning neurological and memory disorders. From the perspective of DNA methylation, the brain is a fascinating organ. It exhibits certain unique features in the following respects- (i) has the highest levels of DNA methylation as compared with other vertebrate organs and tissues except thymus [21] and (ii) has the highest genomic content of 5-hydroxymethylcytosine (5hmC), which is the second-most prevalent DNA methylation mark after the canonical 5-methylcytosine (5mC) [55]. Conventionally, DNA methylation takes place in the context of a dinucleotide sequence CG or a stretch of nucleotides CGGCGG where the cytosine is methylated by the DNMTs. Interestingly, the brain tissues are enriched in DNA methylation in the context mCpH, where H is any nucleotide except G, with mCpH levels increasing rapidly post-partum to mid-adolescence which is a period of active learning [55]. Such unique features led us to explore some aspects of neurological disorders that result as a function of aberrant DNA methylation.

Alzheimer's disease (AD)

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to a progressive impairment of cognition and behavioural functions including memory, comprehension, speaking skills, attentiveness, memory recollection and power of judgement and is the leading cause of dementia worldwide [48]. Recent findings revealed that with increasing age, there is a concomitant decrease of DNA methylation in the genomic DNA. Analysis of post-mortem neural tissue from patients with AD have revealed a significant decrease in DNA methylation levels compared to samples from healthy individuals. This decrease has also been observed to be inversely related to the markers of neurofibrillary tangles in neurons of patients with advanced AD, suggesting that there is a marked loss of DNA methylation in AD patients [63]. Several experiments have elucidated that several proteins that play a role in AD pathogenesis have abnormal methylation patterns in the promoter regions of their corresponding genes. These include among others, Tau and its related genes, Amyloid β (Aβ) and its related genes, apolipoproteins, ribosomal DNA, as well as genes involved in various metabolic pathways [69]. A profiling study on the APP-PSEN1 double transgenic (DTg) mice (these mice show an increased proportion of $A\beta_{42}$ and has an earlier onset and more rapid rate of pathogenesis of AD and serves as animal model of AD [23]) has shown that there is a significant decrease in 5hmC levels in hippocampus but no detectable change in cerebral cortex and cerebellum as the mice aged progressively. This supported the notion that 5hmC-mediated epigenetic regulation could be a potential candidate in the pathogenesis of AD [73].

Fragile X syndrome (FXS)

The monogenic disorder Fragile X Syndrome (FXS) is caused by increased methylation of a tandem repeat sequence CGG in the 5′ UTR of the fragile X mental retardation protein translational regulator 1 (*FMR1*) gene. The encoded protein, Fragile X Mental Retardation Protein (FMRP) binds RNA and is associated with polysomes. FMRP may be involved in mRNA trafficking from the nucleus to the cytoplasm. In the full mutation variant of FXS, more than 200 CGG repeats are identified by the DNMTs in the *FMR1*

locus, while the normal allele has only 6–53 repeats. This leads to the recruitment of MeCP2, which recognizes methylated CpG and calls for HDACs to initiate chromatin condensation. This leads to loss of FMRP expression and subsequent development of intellectual disability and autism-like features. Thus far, there are not targeted cure available for this disease and the exact steps involved in silencing of the gene are yet to be deciphered.

Epilepsy

Epilepsy is one of the most common neurological disorders known to affect humans. The relationship between DNA methylation, pathogenesis and progression of the disease, termed as epileptogenesis, is an emerging field of intense research. Of the many prevalent forms of the disease, temporal lobe epilepsy—hippocampal sclerosis (TLE-HS) has been shown to be linked to the hypermethylation at the *RELN* locus. *RELN* secretes an extracellular glycoprotein that has been linked to controlling cell–cell interactions which are critical for cell positioning and neuronal migration during neural development [47]. The link between hypermethylation and epileptogenesis hypothesis thus proposed states that increased DNMT activity and resultant global genomic hypermethylation that may lead to the progression of the disease as well as maintenance of the diseased state in an affected individual [46]. Interestingly, recent whole genome bisulfite sequencing (WGBS) of the DNA samples collected from relevant patients suffering from TLE-HS type I and TLE without HS, revealed 1171 hypermethylated and 2537 hypomethylated regions, in addition to 632 differentially methylated genes that are primarily involved in epileptogenesis [97]. This further underlined the critical role of DNA methylation as an imperative factor regulating the onset and sub-type of this disease.

Schizophrenia

Schizophrenia is a neurological disorder that affects more than 20 million people worldwide and is characterized by distortions in thinking, perception, emotions, language, sense of self and behavior [WHO Fact Sheet, Schizophrenia (who.int)]. Treatment-resistant Schizophrenia patients are resistant to the most prescribed anti-psychotic medications and clozapine is a second-generation drug that is usually prescribed to these patients [1]. Recent efforts using large scale genome-wide association studies (GWAS) have been successful in identifying local CpG sites having variable methylation patterns (cis-mQTL) that could play a significant role in pathogenesis of the disease [36]. A systematic epigenome-wide association studies (EWAS) to analyse methylation profiles of DNA collected from blood sampled of seven different cohorts have identified 95 differentially methylated positions (DMPs) associated with psychosis and 1048 DMPs associated with treatment-resistant schizophrenia. This study also highlights the identification of potential biomarker candidate genes as well as open future avenues towards successful development of therapeutics [35].

Future clinical prospects

As discussed earlier, DNA methylation is a critical parameter affecting our health and well-being. The current review primarily aims to bring a holistic picture of the crosstalk between environment, diet and lifestyle with DNA methylation. Such studies hold immense potential for therapeutic purposes. Different classes of drugs are used to treat different disorders and the efficacy of these treatments depends on the gene expression characteristic of an individual. Epigenetic regulation on expression pattern of the genes in an individual plays a vital role in this aspect and has given rise to a concept of ‘personalised medicine’ for treatment of various diseases [70]. DNA methylation being highly influenced by the diet and lifestyle patterns of an individual, has presently brought us to the doorstep of a novel concept called nutriepigenomics which has eventually led to the designing of 'epigenetics diet' [72]. Bioactive dietary compounds like isothiocyanates, resveratrol, EGCG, flavonoids, folate and other vitamins, etc. have been proven to affect the epigenome and finetune gene expression. Thus, personalized nutrition can ameliorate predisposition to various degenerative disorders and affect the phenotype of an individual such that the disease treatment might prove to be more efficient [18].

Recent research is directed towards applying the knowledge of how DNA methylation affects Alzheimer’s Disease (AD) to generate biomarkers that would aid in the early detection of AD before the pathogenesis reaches an irreversible end. Correlating changes in DNA methylation levels of peripheral blood mononuclear cells to the cognitive performance in Late Onset AD patients is one such potential method [17]. A promising development on this aspect, comes from the work of Wei et al., who have shown that although DNA methylation patterns between brain and peripheral tissues is differential, DNA methylation levels of the Amyloid Precursor Protein (APP) gene is consistent in both tissues and is a prospective candidate for a viable AD pathogenesis biomarker [82]. The phenotypic manifestation of Fragile X syndrome (FXS) has been correlated with eIF4E phosphorylation in both human and mice patients [31, 37, 75] and currently, the available treatments to modulate eIF4E activity are by the usage of the anti-diabetic drug Metformin [28], the anti-cancer drug eFT508 or tomivosertib [74] among others which have unwanted side effects. Presently for treatment of FXS, efforts are being taken to induce expression of the *FMR1* gene, whose repression results in the disease manifestation. For this DNA methylation

Table 1 Effect of nutrients, dietary supplements, environmental pollutants and lifestyle adaptations on DNA methylation and consequently gene regulation resulting in a plethora of manifestations at different stages of life

Group	Substance	Age	Phenotype	Molecular effects
Methyl group supplements	Betaine or trimethyl glycine	In utero	Affects various features based on regions of hypermethylation—skin pigmentation, tumour suppressor genes, and proto-oncogenes, metabolism specific genes, onset of Type 2 Diabetes mellitus	Global hypermethylation due to SAM generation (15; 83; 88; 89)
Vitamins	Vitamin B9 (Folic Acid)	In utero	Affects features based on region of hypermethylation	Global hypermethylation due to SAM metabolite generation [61]
		Adulthood	Decreases the risk of colorectal cancer	Synthesis of Methionine, SAM for the maintenance of DNA methylation (30; 45; 95).
	Ratio of folate and B12	In utero	B12 deficient and Folate over-supplementation (BDFO) leads to highest mortality rate in mice. Females cannot reproduce	SAM metabolite generation and methylation of CpG islands of DNMT genes affected [61]
	Vitamin B12 (Cyanocobalamin)	In utero	Affects features based on regions of hypermethylation	Global hypermethylation due to SAM metabolite generation [61]
	Vitamin C (Ascorbic Acid)	In utero	Females have more primordial germ cells; effect on fertility of the female reproductive system	Co-factor for TET group of enzymes; increases demethylation for germline specific genes to increase their expression during germline reprogramming [19]
Lipids	High lipid content in maternal diet	In utero	Increase of leptin in cord blood, increases risk of cancer and type 2 diabetes, cholesterol and insulin levels, neurocognitive functions disrupted	The DNA methylation and other histone modification marks remain as epigenetic memory for several genes predisposing the offspring to risk of these disease phenotypes in adulthood (16; 44; 87)
	Palmitic acid, oleic acid, arachidonic acid (AHA), docosahexaenoic acid (DHA) in mother's milk	*Post-partum*	Tendency of obesity in adulthood is lower	Exposure to mother's milk cause a progressive demethylation of Fgf21 and other fatty acid metabolism genes in the offspring liver, reducing the chances of obesity in the offspring [92]
	Omega-3-PUFA	Adulthood	Regulation concentration of HDL-C in blood, associated with cardiovascular health	Decreases the methylation of leucocyte ABCA1 gene [27]
Plant metabolites	EGCG	Adulthood	Reduces the risk of gastric cancers	Inhibitor of methyltransferases; reactivates genes silenced in cancer (CDX2, BMP2) [24]
	DHCA	Adulthood	Increased peripheral inflammatory cytokine Interleukin 6 is associated with Major Depressive Disorder	Decreases the methylation of introns 1 and 3 of the *IL- 6* genes, which act as enhancers [81]
Toxic metals	Arsenic, Mercury, Molybdenum, Lead	In utero	Predisposition to cancer and chromosomal anomalies due to DNA damage	Toxic metal exposure affects the global DNA methylation as well as the promoter methylation of DNA damage repair genes, leading to inefficient DNA damage repair [66]

Table 1 (continued)

Group	Substance	Age	Phenotype	Molecular effects
Environmental pollutants	Diesel exhaust	In utero	Heart related diseases like cardiac hypertrophy, fibrosis and heart failure	Altered methylation of regulatory regions of the DNA leading to aberrant expression of several genes related to heart development [32]
	Bisphenol A from plastic bottles	In utero	Predisposition to neurocognitive disabilities	Exposure leads to an aberrant methylation of *BDNF* gene and affects Bdnf expression levels [49]
Alcohol		Adulthood	Associated with the susceptibility and adaptation to chronic stress; reduction in GDNF expression affects the behavioural responses to chronic stress	Lack of GDNF expression which leads to dysregulation of endogenously controlled alcoholism [2, 4, 20, 62, 99]
Smoking		Adulthood	Associated with immune dysfunction, leading to predisposition to a variety of diseases	Alteration of DNA methylation in genes involved in immune response, haematological pathways, nervous diseases, cancers [76]

inhibitors like 5-aza-2'deoxycytidine and HDAC inhibitors [13], non-coding RNA based therapy and gene therapy using viral vectors [3] are being used. Similarly, therapy for epilepsy is being focused on usage of DNA methylation blockers and HDAC inhibitors, as well as administration of a ketogenic diet, adenosine supplementation or a combination of both [7], although the research is still in its infancy.

The presence of a disease-causing allele in an individual does not necessarily ensure manifestation of the disease. Disease onset and progress actually depends heavily on the epigenetic regulation and consequent gene expression pattern of the mutant allele. Hence, targeting DNA methylation and other associated epigenetic players might open up new avenues of personalized treatment strategies that hold tremendous future potential from a clinical perspective.

Conclusion

In summary, here we have put forth an emerging picture of how diet, changing environment and lifestyle adaptations can influence DNA methylation in the offspring and during adulthood. As presented in Table 1, it is very evident how dietary habits, environmental conditions, social and lifestyle practices intervene our life at various stages, intriguingly at the molecular level. These effects tend to alter our epigenome and resultantly the fidelity of our genomic structure and functions. Such alterations often lead to chronic and dire consequences. Our knowledge in this perspective, however, is still far from complete. We still need to know the exact epigenetic writers/readers/erasers that significantly influence the steps of our life starting from foetus development to adulthood, onset or predisposition to various diseases as life progresses. The quest is still on as to how early an intervention can circumvent such malfunctioning at the epigenetic and genetic levels. With increase in urbanization, frequent environmental changes and exposure to various stressors in life, ensuring proper development before birth and life maintenance thereafter is becoming a growing challenge and consequently, a necessitated area of research.

Acknowledgements This work was supported by Department of Science and Technology, India [DST-FIST Grant No. SR/FST/COLLEGE-014/2010(C)] to St. Xavier's College, Kolkata.

Authors' contributions RNC conceptualised the work; GD, SS, PM and RNC contributed to manuscript preparation. All the authors read and approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare that there are no competing interests associated with the manuscript.

References

1. Ajnakina O, Horsdal HT, Lally J, MacCabe JH, Murray RM, et al. Validation of an algorithm-based definition of treatment resistance in patients with schizophrenia. Schizophr Res. 2018;197:294–7.
2. Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics. 2011;3:267–77.
3. Arsenault J, Gholizadeh S, Niibori Y, Pacey LK, Halder SK, et al. FMRP expression levels in mouse central nervous system neurons determine behavioral phenotype. Hum Gene Ther. 2016;27:982–96.
4. Barak S, Ahmadiantehrani S, Logrip ML, Ron D. GDNF and alcohol use disorder. Addict Biol. 2019;24:335–43.
5. Beausoleil C, Emond C, Cravedi JP, Antignac JP, Applanat M, et al. Regulatory identification of BPA as an endocrine disruptor: context and methodology. Mol Cell Endocrinol. 2018;475:4–9.
6. Bestor TH. The DNA methyltransferases of mammals. Hum Mol Genet. 2000;9:2395–402.
7. Boison D, Rho JM. Epigenetics and epilepsy prevention: The therapeutic potential of adenosine and metabolic therapies. Neuropharmacology. 2020;167:107741.
8. Cedernaes J, Schonke M, Westholm JO, Mi J, Chibalin A, et al. Acute sleep loss results in tissue-specific alterations in genome-wide DNA methylation state and metabolic fuel utilization in humans. Sci Adv. 2018;4:eaar8590.
9. Chavez-Lizarraga D, Zarate-Segura P, Beltran-Montoya J, Canchola-Sotelo C, Vadillo-Ortega F, Chavira-Suarez E. DNA methylation variability in a single locus of the RXRalpha promoter from umbilical vein blood at term pregnancy. Biochem Genet. 2018;56:210–24.
10. Chen B, Wu L, Cao T, Zheng HM, He T. MiR-221/SIRT1/Nrf2 signal axis regulates high glucose induced apoptosis in human retinal microvascular endothelial cells. BMC Ophthalmol. 2020;20:300.
11. Chen D, Meng L, Pei F, Zheng Y, Leng J. A review of DNA methylation in depression. J Clin Neurosci. 2017;43:39–46.
12. Chen J, Haanpaa MK, Gruber JJ, Jager N, Ford JM, Snyder MP. High-resolution bisulfite-sequencing of peripheral blood DNA methylation in early-onset and familial risk breast cancer patients. Clin Cancer Res. 2019;25:5301–14.
13. Chiurazzi P, Pomponi MG, Pietrobono R, Bakker CE, Neri G, Oostra BA. Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene. Hum Mol Genet. 1999;8:2317–23.
14. Chmurzynska A, Mlodzik MA, Radziejewska A, Szwengiel A, Malinowska AM, Nowacka-Woszuk J. Caloric restriction can affect one-carbon metabolism during pregnancy in the rat: a transgenerational model. Biochimie. 2018;152:181–7.
15. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr. 2002;132:2393S-S2400.
16. Daniels TE, Sadovnikoff AI, Ridout KK, Lesseur C, Marsit CJ, Tyrka AR. Associations of maternal diet and placenta leptin methylation. Mol Cell Endocrinol. 2020;505:110739.
17. Di Francesco A, Arosio B, Falconi A, Micioni Di Bonaventura MV, Karimi M, et al. Global changes in DNA methylation in Alzheimer's disease peripheral blood mononuclear cells. Brain Behav Immun. 2015;45:139–44.
18. Di Renzo L, Gualtieri P, Romano L, Marrone G, Noce A, et al. Role of personalized nutrition in chronic-degenerative diseases. Nutrients. 2019;11:1968.
19. DiTroia SP, Percharde M, Guerquin MJ, Wall E, Collignon E, et al. Maternal vitamin C regulates reprogramming of DNA methylation and germline development. Nature. 2019;573:271–5.
20. Dogan MV, Lei MK, Beach SR, Brody GH, Philibert RA. Alcohol and tobacco consumption alter hypothalamic pituitary adrenal axis DNA methylation. Psychoneuroendocrinology. 2016;66:176–84.
21. Ehrlich M, Gama-Sosa MA, Huang LH, Midgett RM, Kuo KC, et al. Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. Nucleic Acids Res. 1982;10:2709–21.
22. Elliott E, Ezra-Nevo G, Regev L, Neufeld-Cohen A, Chen A. Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. Nat Neurosci. 2010;13:1351–3.
23. Esquerda-Canals G, Montoliu-Gaya L, Guell-Bosch J, Villegas S. Mouse models of Alzheimer's disease. J Alzheimers Dis. 2017;57:1171–83.
24. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, et al. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res. 2003;63:7563–70.
25. Fu Y, Liu M, Li F, Qian L, Zhang P, et al. MiR-221 Promotes Hepatocellular Carcinoma Cells Migration via Targeting PHF2. Biomed Res Int. 2019;2019:4371405.
26. Fuchikami M, Morinobu S, Segawa M, Okamoto Y, Yamawaki S, et al. DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. PLoS One. 2011;6:e23881.
27. Fujii R, Yamada H, Munetsuna E, Yamazaki M, Mizuno G, et al. Dietary fish and omega-3 polyunsaturated fatty acids are associated with leukocyte ABCA1 DNA methylation levels. Nutrition. 2021;81:110951.
28. Gantois I, Khoutorsky A, Popic J, Aguilar-Valles A, Freemantle E, et al. Metformin ameliorates core deficits in a mouse model of fragile X syndrome. Nat Med. 2017;23:674–7.
29. Ghoshal K, Li X, Datta J, Bai S, Pogribny I, et al. A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. J Nutr. 2006;136:1522–7.
30. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr. 2002;132:2350S-S2355.
31. Gkogkas CG, Khoutorsky A, Cao R, Jafarnejad SM, Prager-Khoutorsky M, et al. Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell Rep. 2014;9:1742–55.
32. Goodson JM, MacDonald JW, Bammler TK, Chien WM, Chin MT. In utero exposure to diesel exhaust is associated with alterations in neonatal cardiomyocyte transcription, DNA methylation and metabolic perturbation. Part Fibre Toxicol. 2019;16:17.
33. Gruenbaum Y, Cedar H, Razin A. Substrate and sequence specificity of a eukaryotic DNA methylase. Nature. 1982;295:620–2.
34. Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell. 2011;145:423–34.
35. Hannon E, Dempster EL, Mansell G, Burrage J, Bass N, et al. DNA methylation meta-analysis reveals cellular alterations in psychosis and markers of treatment-resistant schizophrenia. Elife. 2021;10:98.
36. Hannon E, Spiers H, Viana J, Pidsley R, Burrage J, et al. Methylation QTLs in the developing brain and their enrichment in schizophrenia risk loci. Nat Neurosci. 2016;19:48–54.
37. Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E. Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. Neuron. 2006;51:441–54.
38. Huang RC, Galati JC, Burrows S, Beilin LJ, Li X, et al. DNA methylation of the IGF2/H19 imprinting control region and adiposity distribution in young adults. Clin Epigenetics. 2012;4:21.

39. Humphreys KL, Moore SR, Davis EG, MacIsaac JL, Lin DTS, et al. DNA methylation of HPA-axis genes and the onset of major depressive disorder in adolescent girls: a prospective analysis. Transl Psychiatry. 2019;9:245.
40. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010;466:1129–33.
41. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr. 1998;128:1204–12.
42. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, et al. Epigenetic signatures of cigarette smoking. Circ Cardiovasc Genet. 2016;9:436–47.
43. Kadayifci FZ, Zheng S, Pan YX. Molecular mechanisms underlying the link between diet and DNA methylation. Int J Mol Sci. 2018;19:23.
44. Keleher MR, Zaidi R, Shah S, Oakley ME, Pavlatos C, et al. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. PLoS One. 2018;13:e0192606.
45. Kim MS, Lee J, Sidransky D. DNA methylation markers in colorectal cancer. Cancer Metastasis Rev. 2010;29:181–206.
46. Kobow K, Blümcke I. The methylation hypothesis: Do epigenetic chromatin modifications play a role in epileptogenesis? Epilepsia. 2011;52:15–9.
47. Kobow K, Jeske I, Hildebrandt M, Hauke J, Hahnen E, et al. Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. J Neuropathol Exp Neurol. 2009;68:356–64.
48. Kumar A, Sidhu J, Goyal A, Tsao JW. Alzheimer Disease. In *StatPearls*. Treasure Island (FL). Number of, 2021.
49. Kundakovic M, Gudsnuk K, Herbstman JB, Tang D, Perera FP, Champagne FA. DNA methylation of BDNF as a biomarker of early-life adversity. Proc Natl Acad Sci U S A. 2015;112:6807–13.
50. Lang Y, Li Y, Yu H, Lin L, Chen X, et al. HOTAIR drives autophagy in midbrain dopaminergic neurons in the substantia nigra compacta in a mouse model of Parkinson's disease by elevating NPTX2 via miR-221-3p binding. Aging (Albany NY). 2020;12:7660–78.
51. Lawson HA, Cheverud JM, Wolf JB. Genomic imprinting and parent-of-origin effects on complex traits. Nat Rev Genet. 2013;14:609–17.
52. Li H, Niswander L. Does DNA methylation provide a link between folate and neural tube closure? Epigenomics. 2018;10:1263–5.
53. Li L, Wang Q, Zhang Y, Niu Y, Yao X, Liu H. The molecular mechanism of bisphenol A (BPA) as an endocrine disruptor by interacting with nuclear receptors: insights from molecular dynamics (MD) simulations. PLoS One. 2015;10:e0120330.
54. Li N, Zhou H, Tang Q. miR-133: a suppressor of cardiac remodeling? Front Pharmacol. 2018;9:903.
55. Lister R, Mukamel EA. Turning over DNA methylation in the mind. Front Neurosci. 2015;9:252.
56. Liu H, Chang JK, Hou JQ, Zhao ZH, Zhang LD. Inhibition of miR-221 influences bladder cancer cell proliferation and apoptosis. Eur Rev Med Pharmacol Sci. 2020;24:7550.
57. Long HK, King HW, Patient RK, Odom DT, Klose RJ. Protection of CpG islands from DNA methylation is DNA-encoded and evolutionarily conserved. Nucleic Acids Res. 2016;44:6693–706.
58. Lopez-Sanchez C, Franco D, Bonet F, Garcia-Lopez V, Aranega A, Garcia-Martinez V. Reciprocal repression between Fgf8 and miR-133 regulates cardiac induction through Bmp2 signaling. Data Brief. 2015;5:59–64.
59. Lussier AA, Bodnar TS, Mingay M, Morin AM, Hirst M, et al. Prenatal alcohol exposure: profiling developmental DNA methylation patterns in central and peripheral tissues. Front Genet. 2018;9:610.
60. Ma K, Zhao C, Guo K, Fu Z, Che C, et al. Low HOXC10 expression in liver cancer regulates proliferation via a mechanism involving miR-221 and the MAPK signaling pathway. Oncol Lett. 2020;20:127.
61. Mahajan A, Sapehia D, Thakur S, Mohanraj PS, Bagga R, Kaur J. Effect of imbalance in folate and vitamin B12 in maternal/parental diet on global methylation and regulatory miRNAs. Sci Rep. 2019;9:17602.
62. Maier HB, Neyazi M, Neyazi A, Hillemacher T, Pathak H, et al. Alcohol consumption alters Gdnf promoter methylation and expression in rats. J Psychiatr Res. 2020;121:1–9.
63. Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD, Rogers J. Epigenetic changes in Alzheimer's disease: decrements in DNA methylation. Neurobiol Aging. 2010;31:2025–37.
64. Maud C, Ryan J, McIntosh JE, Olsson CA. The role of oxytocin receptor gene (OXTR) DNA methylation (DNAm) in human social and emotional functioning: a systematic narrative review. BMC Psychiatry. 2018;18:154.
65. Mok GF, Lozano-Velasco E, Maniou E, Viaut C, Moxon S, et al. miR-133-mediated regulation of the Hedgehog pathway orchestrates embryo myogenesis. Development. 2018;145:1666.
66. Montes-Castro N, Alvarado-Cruz I, Torres-Sanchez L, Garcia-Aguiar I, Barrera-Hernandez A, et al. Prenatal exposure to metals modified DNA methylation and the expression of antioxidant- and DNA defense-related genes in newborns in an urban area. J Trace Elem Med Biol. 2019;55:110–20.
67. Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet. 1998;19:219–20.
68. Pidsley R, Dempster E, Troakes C, Al-Sarraj S, Mill J. Epigenetic and genetic variation at the IGF2/H19 imprinting control region on 11p15.5 is associated with cerebellum weight. Epigenetics. 2012;7:155–63.
69. Qazi TJ, Quan Z, Mir A, Qing H. Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation. Mol Neurobiol. 2018;55:1026–44.
70. Rasool M, Malik A, Naseer MI, Manan A, Ansari S, et al. The role of epigenetics in personalized medicine: challenges and opportunities. BMC Med Genomics. 2015;8(Suppl 1):S5.
71. Ravegnini G, Cargnin S, Sammarini G, Zanotti F, Bermejo JL, et al. Prognostic role of miR-221 and miR-222 expression in cancer patients: a systematic review and meta-analysis. Cancers (Basel). 2019;11:10235.
72. Remely M, Stefanska B, Lovrecic L, Magnet U, Haslberger AG. Nutriepigenomics: the role of nutrition in epigenetic control of human diseases. Curr Opin Clin Nutr Metab Care. 2015;18:328–33.
73. Shu L, Sun W, Li L, Xu Z, Lin L, et al. Genome-wide alteration of 5-hydroxymenthylcytosine in a mouse model of Alzheimer's disease. BMC Genom. 2016;17:381.
74. Shukla T, de la Pena JB, Perish JM, Ploski JE, Stumpf CR, et al. A highly selective MNK inhibitor rescues deficits associated with Fragile × Syndrome in Mice. Neurotherapeutics. 2021;18:624–39.
75. Sidhu H, Dansie LE, Hickmott PW, Ethell DW, Ethell IM. Genetic removal of matrix metalloproteinase 9 rescues the symptoms of fragile × syndrome in a mouse model. J Neurosci. 2014;34:9867–79.
76. Su D, Wang X, Campbell MR, Porter DK, Pittman GS, et al. Distinct epigenetic effects of tobacco smoking in whole blood and among leukocyte subtypes. PLoS One. 2016;11:e0166486.

77. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324:930–5.
78. Tanaka M, Ito S, Matsushita N, Mori N, Kiuchi K. Promoter analysis and characteristics of the 5'-untranslated region of the mouse glial cell line-derived neurotrophic factor gene. Brain Res Mol Brain Res. 2000;85:91–102.
79. Tiraboschi RB, Neto FSL, da Cunha TDP, de Bessa J, Jr., Miranda EP, , et al. Expression of MicroRNAs (miR-15b, miR-16, miR-138, miR-221, and miR-222) as biomarkers of endothelial corpus cavernosum dysfunction in a diabetic alcoholic murine model. Sex Med. 2021;9:100326.
80. Van den Wyngaert I, Sprengel J, Kass SU, Luyten WH. Cloning and analysis of a novel human putative DNA methyltransferase. FEBS Lett. 1998;426:283–9.
81. Wang J, Hodes GE, Zhang H, Zhang S, Zhao W, et al. Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. Nat Commun. 2018;9:477.
82. Wei X, Zhang L, Zeng Y. DNA methylation in Alzheimer's disease: In brain and peripheral blood. Mech Ageing Dev. 2020;191:111319.
83. Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J. 1998;12:949–57.
84. Xiang N, Zhao R, Song G, Zhong W. Selenite reactivates silenced genes by modifying DNA methylation and histones in prostate cancer cells. Carcinogenesis. 2008;29:2175–81.
85. Xu C, Lu Y, Pan Z, Chu W, Luo X, et al. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. J Cell Sci. 2007;120:3045–52.
86. Xu P, Xin J, Song L, Chen Y, Ma J, et al. Serum miR-133 as a potential biomarker in acute cerebral infarction patients. Clin Lab. 2020;66:398.
87. Yan W, Zhang Y, Wang L, Yang W, Li C, et al. Maternal dietary glycaemic change during gestation influences insulin-related gene methylation in the placental tissue: a genome-wide methylation analysis. Genes Nutr. 2019;14:17.
88. Yang S, Zhao N, Sun B, Yang Y, Hu Y, Zhao R. Grandmaternal betaine supplementation enhances hepatic IGF2 expression in F2 rat offspring through modification of promoter DNA methylation. J Sci Food Agric. 2020;100:1486–94.
89. Yang S, Zhao N, Yang Y, Hu Y, Dong H, Zhao R. Mitotically stable modification of DNA methylation in IGF2/H19 imprinting control region is associated with activated Hepatic IGF2 expression in offspring rats from betaine-supplemented dams. J Agric Food Chem. 2018;66:2704–13.
90. Yasser MB, Abdellatif M, Emad E, Jafer A, Ahmed S, et al. Circulatory miR-221 & miR-542 expression profiles as potential molecular biomarkers in Hepatitis C Virus mediated liver cirrhosis and hepatocellular carcinoma. Virus Res. 2021;296:198341.
91. Yoder JA, Bestor TH. A candidate mammalian DNA methyltransferase related to pmt1p of fission yeast. Hum Mol Genet. 1998;7:279–84.
92. Yuan X, Tsujimoto K, Hashimoto K, Kawahori K, Hanzawa N, et al. Epigenetic modulation of Fgf21 in the perinatal mouse liver ameliorates diet-induced obesity in adulthood. Nat Commun. 2018;9:636.
93. Yuasa Y, Nagasaki H, Akiyama Y, Hashimoto Y, Takizawa T, et al. DNA methylation status is inversely correlated with green tea intake and physical activity in gastric cancer patients. Int J Cancer. 2009;124:2677–82.
94. Yuasa Y, Nagasaki H, Akiyama Y, Sakai H, Nakajima T, et al. Relationship between CDX2 gene methylation and dietary factors in gastric cancer patients. Carcinogenesis. 2005;26:193–200.
95. Zhan YX, Luo GH. DNA methylation detection methods used in colorectal cancer. World J Clin Cases. 2019;7:2916–29.
96. Zhang H, Wang L, Peng F, Wang X, Gong H. L-arginine ameliorates high-fat diet-induced atherosclerosis by downregulating miR-221. Biomed Res Int. 2020;2020:4291327.
97. Zhang W, Wang H, Liu B, Jiang M, Gu Y, et al. Differential DNA methylation profiles in patients with temporal lobe epilepsy and hippocampal sclerosis ILAE type I. J Mol Neurosci. 2021;3:69.
98. Zhang XT, Zhang Z, Xin YN, Ma XZ, Xuan SY. Impairment of growth of gastric carcinoma by miR-133-mediated Her-2 inhibition. Tumour Biol. 2015;36:8925–30.
99. Zhang Y, Wang L, Wang X, Wang Y, Li C, Zhu X. Alterations of DNA methylation at GDNF gene promoter in the ventral tegmental area of adult depression-like rats induced by maternal deprivation. Front Psychiatry. 2018;9:732.
100. Zhao H, Yan P, Wang J, Zhang Y, Zhang M, et al. Clinical significance of tumor miR-21, miR-221, miR-143, and miR-106a as biomarkers in patients with osteosarcoma. Int J Biol Markers. 2019;34:184–93.
101. Zheng ZZ, Ma YP, Wu RH, Rong G, Li C, et al. Serum miR-133 as a novel biomarker for predicting treatment response and survival in acute myeloid leukemia. Eur Rev Med Pharmacol Sci. 2020;24:777–83.