Chapter

Global DNA Methylation as a Potential Underlying Mechanism of Congenital Disease Development

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Abstract

During the last decade, quantitative measurement of the methylation status in white blood cells (WBCs) has been used as a potential biomarker in a variety of diseases. Long interspersed nucleotide element-1 (LINE-1) has been used widely as a surrogate marker of global DNA methylation. Altered maternal DNA methylation is suggested to be an underlying mechanism in the trisomy 21 and the development of birth defects, including congenital heart defects (CHDs). The molecular mechanisms that underlie the epigenetic regulation of gene transcription are independent of DNA sequence, but they do depend on environmental stimuli, which are especially important in fetal development in utero environment. Folic acid deficiency and genetic variations of folate pathway genes, such as the methylenetetrahydrofolate reductase gene (*MTHFR*), are foremost among these maternal risk factors. Also, there are exogenous risk factors (cigarette smoking, alcohol intake, medication use, periconceptional maternal supplementation, body mass index, and dietary habits) with impact on maternal LINE-1 methylation. *MTHFR* C677T genotype/diet and other environmental factors as significant predictors of LINE-1 DNA methylation in regard to congenital diseases will be discussed in the chapter.

Keywords: DNA methylation, LINE-1, congenital anomaly, development, nutrition, folate intake, genotype

1. Introduction

According to WHO, congenital anomalies (CAs) are birth defects that can be defined as structural or functional malformations [1]. CAs occur during intrauterine life and can be identified prenatally or at birth or later in infancy. CAs are important causes of infant and childhood deaths and chronic illness/disability. Long-term disability may have significant impacts on patients, families, healthcare systems, and societies. Some CAs can be prevented by adequate intake of folic acid (FA) through fortification of staple foods or supplementation. Among severe congenital anomalies, the most common ones are congenital heart defects (CHDs), neural tube defects (NTD), and Down syndrome (DS). For renal dysplasia an increasing trend was observed recently in Europe [2]. Several factors have been proposed to have a significant role in the development of CAs: one or more genes;

infectious, maternal diabetes or obesity; and nutritional and environmental factors [2]. Identification of the exact cause/causes recently became even more complicated with addition of new factors. Epigenetic factors, as it is DNA methylation, have been shown to have an impact on the gene expression, through modulation by nutrition or environmental stimuli that occur during intrauterine development, but could even be a consequence of maternal or paternal lifestyle factors. Altered DNA methylation was suggested to be an underlying mechanism in the development of CAs, CHDs, NTD, congenital anomaly of the kidney and urinary tract (CAKUT), and autism spectrum disorders (ASD) and in imprinting genetic disorders [3–12]. Congenital heart defects (CHDs) are the most common birth defects in humans with a prevalence of 0.8% [13, 14]. Only about 15–20% of CHDs can be attributed to known causes, whereas chromosomal abnormalities occur in 5–10% of cases [14]. The highest association with major heart abnormalities is observed in DS [15]. CHDs are reported in approximately 40% of DS cases, typically involving septal defects such as atrial septal defect, ventricular septal defect, and complete atrioventricular canal [16]. The etiology of most CHDs remains largely unknown, but it is considered to involve multiple genetic, epigenetic, environmental, and lifestyle factors [13, 14, 17]. Risk factors, including aging, body mass index (BMI), cigarette smoking, alcohol intake, folate deficiency, MTHFR polymorphisms, and hyperhomocysteinemia, have been proposed to be the modulators of DNA methylation patterns [3–6, 18–20]. Maternal intrauterine milieu, such as maternal environment during pregnancy (hypoxia, stress, obesity, diabetes, toxins, altered nutrition, inflammation, and reduced utero-placental blood flow) could affect fetal methylation programming, thereby affecting fetal growth and the lifelong health of the fetus [21, 22]. It was reported that the maternal LINE-1 hypomethylation is linked with the increased risk for non-syndromic CHD, particularly septal defects [4, 5].

2. DNA gene-specific methylation and global DNA methylation

DNA methylation is a key factor of the epigenetic machinery that is responsible for regulating gene expression and, therefore, cell function. This component is one of the most important in mammalian embryonic development, differentiation, and many of congenital and complex diseases [3–6, 23–25]. The DNA methylation has nonrandom, well-regulated, and tissue-specific patterns [26]. Abnormal gene-specific demethylation and global hypomethylation (involving repeat sequences throughout the genome) can potentially lead to overexpression of genes and activation of transposable elements contributing to disease. Regulation of gene expression through methylated or unmethylated human genome can exist at approximately 3×10^7 CpG short sequences of 5–10 CpG dinucleotides [27, 28].

DNA methylation is required in many processes such as X chromosome inactivation, imprinting, embryogenesis, gametogenesis, and silencing of repetitive DNA elements [29]. It refers to the covalent addition of a methyl group to the cytosine located at the 5′-position to guanosine in a CpG dinucleotide, catalyzed by the activity of three DNA methyltransferases (DNMTs) [30]. Recent findings of tissue-specific expression of ten-eleven translocation (TET) proteins revealed that this epigenetic event is not irreversible and, even more, TET was shown to be able to modify methylcytosine and potentially erase DNA methylation [31].

Each of the three DNMT genes was found to be mutated in specific and diverse human syndromes [32]. DNA methylation is required to protect chromosomal integrity, by preventing reactivation of endoparasitic sequences that cause chromosomal instability, translocations, increased mutation events, loss of imprinting, and gene disruption [29]. Genome-wide methylation profiling has recently become

possible and revealed genes of interest that were enriched in multiple biological processes involved in fetal development [3], and specific hypermethylation was linked to gene silencing in some pediatric disorders [33, 34]. Moreover, epigenetic mechanisms including parent of origin-specific DNA methylation include genomic imprinting as restriction of gene expression [35]. Moreover, imprinting in embryos was found to be parentally sex-specific, and this effect could be more complex than previously suggested [36]. Hypomethylation of imprinted loci (HIL) throughout the genome was observed in patients with imprinted disorders. Among approximately 70 known imprinted genes, there are some that are causing disorders affecting growth, including one in the DS critical region [35]. Aberrant methylation in four maternally methylated regions was observed at whole genome methylation analysis. However, methylation of a CpG island does not necessarily lead to gene silencing. For example, the gene for telomerase has been shown to be activated by methylation [37]. Telomerases are crucial elements in maintaining cell life, could possibly reverse an aging mechanism, and rejuvenate cell viability. Enzyme telomerase modulates elongation of telomeres, by adding repeating DNA sequences to the ends of the chromosomes, and telomere serves as a bioprotective mechanism of chromosome attrition at each cell division [38]. Telomeres could become too short to allow replication or dysfunctional in some congenital disease which may lead to chromosome instability or cell death [39]. Besides DNA coding region, studies have shown that DNA methylation of noncoding DNA plays an important role in modulating structure and dynamics of chromatin, as well as many other chromatin-dependent processes and their associated biological functions [27].

2.1 LINE-1 DNA methylation

Gene-specific DNA methylation analysis does not provide a global picture of DNA methylation changes within a genome. Global DNA hypomethylation occurs mainly at heavily methylated noncoding regions of DNA, particularly repeat sequences and transposable elements [40, 41].

In humans, nearly 80% CpG islands occur in transposon-derived sequences, throughout the genome, such as long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) [42]. LINE-1 is the largest member of the LINE family with more than 500,000 copies comprising approximately 17% of the genome [43]. CpG islands within LINE-1 sequences and their methylation levels correlate with the global genomic DNA methylation level [44, 45], so LINE-1 methylation has been widely used as a surrogate marker of global genomic DNA methylation [46], and methylation status of LINE-1 in white blood cells (WBC) is a potential biomarker in a variety of diseases [4, 45–48] in research on cancer, cardiovascular, neurodegenerative, and CAs [3–6, 48–51]. Human genome has on average 80–100 active LINE-1, and it has been estimated that new LINE-1 insertion in genome occurs in at least 1 in every 50 humans within a parental germ cell or during early fetal development [40]. Thus, LINE-1 hypomethylation in the parental germline, along with altered miRNA expression, might also significantly affect genome stability during the fetal development [52, 53].

3. DNA methylation during gametogenesis and embryogenesis

DNA methylation changes are particularly dynamic in gametogenesis and early embryogenesis. During the course of mammalian differentiation and development, DNA methylation undergoes remodeling to eventually generate the cell type-specific methylation patterns, found in somatic cells of adults. During the

gametogenesis, DNA is demethylated within each developing germ cell and then remethylated/reset to the methylation patterns specific to gametogenesis. The differentially methylated regions (DMRs) are sperm and egg specific [54, 55]. This process establishing the specific methylation of imprinted loci before fertilization, as well as other non-imprinted loci, may also be subject to at least partial erasure of methylation during gametogenesis [56–58]. The zygotic DNA demethylation after fertilization in mouse embryogenesis affects parental genome on a genome-wide level including single gene loci and repetitive elements. The maternal genome-wide methylation is unaffected [59]. This process changes the methylation patterns of the gametes and establishes the DNA methylation patterns found in somatic differentiated cells in adults through induced expression of DNMT and de novo methylation of genome in post-implantation mouse embryos [60–62]. It has been shown that in small studies of human embryos, there is a demethylation process at the 4-cell stage followed by remethylation at late morula [63]. Even more, expression patterns of DNMTs after cryopreservation of human embryos could be disturbed and could have long-term developmental consequences [64] that suggest the importance of DNA methylation program maintenance during development. Periods during gametogenesis and embryogenesis may also present windows of opportunity for environmental influences on DNA methylation pattern. The DMRs are established during gametogenesis at imprinted and non-imprinted loci and are susceptible to environmental factors [65, 66]. LINE-1 methylation in sperm could be a risk marker of infertility in man at nicotine/alcohol exposure [67]. It is also possible to alter DNA methylation levels and patterns within intact mammalian cells by treatment with various chemical inhibitors, DNA-demethylating drugs, which have recently been introduced as potential therapeutic agents for the treatment of human diseases, particularly myelodysplastic syndromes [68].

The dynamic reprogramming and other epigenetic patterns which could affect normal patterns of gene expression/genome stability during development could lead to an increased risk of CAs or complex diseases later in life [65–67].

4. LINE-1 DNA methylation and environmental influences (e.g., diet and nutrition)

Previous research was focused on the effect of specific foods on the DNA methylation process, but there is currently growing interest in determining how dietary patterns may affect global and local DNA methylation in humans. There are some studies that suggest that frequent use of vegetables and/or fruits decreased the risk of LINE-1 hypomethylation [69–71]. Biological explanation could be in beneficial modulation of pathways involved in epigenetic mechanisms by intake of high variety of nutritive and bioactive substances included in fruit- and vegetable-rich food. These components were polyphenols; flavonoids; carotenoids; folates; vitamins C, E, and A; minerals; and fibers [72, 73]. As it is known that many crucial cellular processes depend on folate, including DNA methylation [74], low folate intake in daily food could be supplemented by synthetic form as folic acid (FA) and through fortification programs [75]. Even more, harmful effect of particulate matter exposure on LINE-1 methylation level could be counteracted by healthy food consumption such as Mediterranean diet [76]. Also, fatty acids can modify DNA methylation in vitro, but limited information is available from human studies. Some studies observed that intake of vegetable oil/dietary fat seemed to be negatively correlated with LINE-1 methylation [69, 77]. Others show no changes in methylation profile after supplementation with grape seed flavanols [78]. The interindividual variation in blood cell DNA methylation in interventional studies, which are usually

rather small, demands studies with larger sample size to avoid masking the possibly subtle changes in DNA methylation in response to dietary factors.

4.1 Methylenetetrahydrofolate reductase (MTHFR), folate metabolism, and its role in DNA methylation

Folate can be a limiting factor in many biological reactions. The methylene tetrahydrofolate reductase (MTHFR) is an enzyme important for the folate metabolism which is in the basis of the DNA, RNA, and protein methylation. Genomic DNA methylation directly correlates with folate status and inversely with plasma homocysteine (tHcy) levels [79–82]. The one-carbon pathway and thus DNA methylation function under tight regulatory controls. S-Adenosyl methionine (SAM) is the major regulator of folate-dependent Hcy remethylation because it is a potent inhibitor of MTHFR. When the SAM concentration is high, MTHFR is inhibited and hence remethylation of homocysteine. Conversely, if SAM concentrations are low, remethylation of homocysteine is favored. Hyperhomocysteinemia is an emerging risk factor for various cardiovascular diseases, and, with the increasing significance of this genetic variant in the view of morbidity and mortality impact on the patients, further prevention strategies and nutritional recommendations with the supplementation of folate would be necessary as part of future health education. Other essential nutrients that are naturally present in some foods or as dietary supplement, like vitamin B_6, B_{12}, B_2, and choline, are necessary in addition to folate to maintain DNA methylation [83]. It is also recognized that S-adenosylhomocysteine (SAH) functions as a potent product inhibitor of SAM-dependent methyltransferases [84]. For this reason, continual hydrolysis of SAH to homocysteine is important for DNA methylation [85]. Plasma homocysteine elevation has been associated with increased concentration of SAH, and increased SAH was in correlation with global DNA hypomethylation [86]. Methionine is the substrate for SAM, a cofactor and methyl group donor for numerous methylation reactions including the methylation of DNA, RNA, and histones [87]. A number of SAM-dependent reactions have regulatory roles by affecting both, genome stability and gene transcription [88].

4.2 Epigenetic, genetic, and nutrigenomic risk factors for congenital diseases: DNA methylation, global DNA methylation, miRNA, MTHFR polymorphism, and low folate status

Low folate status (as defined by various measures including blood folate concentrations, folate intake, and/or FA intake) has been associated with an increased risk of cardiovascular disease, cancers, CAs, CHD, and NTD [5, 6, 89–94]. Also, this deficiency is clearly detrimental to the embryo and shows possible longer-term risks of diabetes or other health outcomes and health problems associated with child mortality and morbidity [95]. Periconceptional supplementation of FA also reduces the risk of congenital heart diseases (previous ref) and preterm birth and low birth weight [96, 97]. The prevalence of neural tube defects (NTDs) has been significantly lowered in more than 70 countries worldwide by applying fortification with FA, but in all European governments there is still an issue with FA fortification of centrally processed and widely eaten foods in prevention of unwanted birth outcomes [98]. The mechanisms by which low folate status contributes to these disorders have not been understood completely but, to a certain extent, could be explained by different molecular pathways. Folate depletion could be a destabilizing factor during DNA replication. If inadequate folate availability is present during cell division, the production of

thymidine could be compromised and may be substituted in the DNA sequence by uracil. This mutagenic event may trigger the defect in an effort to repair DNA and increase the frequency of chromosomal breaks [90]. Low FA in tissue culture has been shown to result in the formation of micronuclei (chromosome breakage) and that the presence of MTHFR C677T polymorphism (TT genotype) increases the micronuclei formation, under the low folate conditions [99]. This MTHFR polymorphism was associated with various diseases, and allele frequencies vary depending on ethnicity (reviewed in [100]). This gene is mapped on chromosome 1 (1p36.6), and the genetic variant assigned as C677T (rs1801133) is located in exon 4 in this gene. This polymorphism results in the conversion at codon 222, valine to alanine. Carriers of the T allele have lower enzyme activity [101]. The MTHFR 677TT homozygous subjects have higher homocysteine levels than the normal, non-mutated controls. To date, most studies have shown that the *MTHFR* C677T genotype is related to biomarkers, such as serum folate, tHcy concentration, and folate intake. Elevated blood tHcy is a well-recognized and modifiable risk factor for cerebral and cardiovascular disease [101, 102]. Reduction of the enzyme activity leads to elevated Hcy concentrations [103]. The TT genotype has been associated with elevated tHcy levels in populations with low folate intake [104]. Previous tHcy-lowering trials have not considered whether and to what extent these factors could modify the efficacy of folic acid (FA) treatment. In some countries with folate fortification like America, Australia, and New Zealand, the effect of TT genotype is not so obvious like in Asia region where folate intake is low [94]. In those who are homozygous for the mutation (TT genotype), enzyme function is only 30% of normal, and data provide evidence that nutrition can counteract genetic susceptibility. Recently, large, randomized trial in a population without mandatory FA fortification demonstrated that the adverse effect of the TT genotype on tHcy levels can be ameliorated by raising serum folate levels above the threshold (15 ng/mg or 34 nmol/L) via FA treatment and it provides new evidence to support a personalized FA treatment [94]. The gene-nutrient interaction between MTHFR C677T variant and folate status was also observed on the risk of anencephaly. Mothers with 677TT genotype with serum folate levels in the upper tercile (>14.1 ng/ml) had a 95% lower risk to have a child with anencephaly than mothers with serum folate levels in the first and second terciles [92]. Results about DS and MTHFRC677T polymorphism as a risk factor of its occurrence are still conflicting. The recent meta-analysis suggested that MTHFR 677T is a major risk factor for DS birth [105], while previous smaller studies did not recognize such risk [106, 107]. Studies performed analyzing peripheral lymphocytes of women with DS offspring revealed several markers of global genome instability, including an increased frequency of micronuclei, shorter telomeres, and impaired DNA methylation at MTHFR promoter [108, 109]. Hypermethylation of MTHFR promoter may lead to CHD in DS subjects [109]. Functional inactivation of MTHFR gene expression could be a mechanism of impaired folate metabolism, which is known to play a role in chromosomal breakage, abnormal chromosomal segregation, and genomic instability and therefore a developmental defect in the CHD in DS. Another suggested mechanism is lower LINE-1 methylation, the surrogate marker for global methylation levels, in young mothers of DS compared to controls, suggesting the possibility of impaired DNA methylation causing maternally derived trisomy 21 [6]. Also, there is evidence from intervention studies of the effects of dietary factors, where FA was the most common intervention agent (33%). Meta-regression analysis showed that the dose of supplementary FA was the only identified factor ($p < 0.001$) showing a positive relationship with DNA methylation patterns in humans [93]. MTHFR genotype-dependent association between lower global DNA methylation and lower plasma folate concentration

was detected in observational studies in healthy subjects [81, 82, 110]. Global DNA methylation at maternal front ($p = 0.04$) and hypomethylation of MTHFR gene at fetal front ($p = 0.001$) might be a characteristic of preeclampsia [111]. The combination of MTHFR C677T genotype and diet significantly influenced global DNA methylation in mothers with DS children. The lowest values of global DNA methylation were observed in mothers with MTHFR 677 CT+TT genotype and low dietary folate [6]. Even more, recently the association between maternal LINE-1 methylation and the occurrence of CHD in children with DS was shown, as well as the impact of endogenous maternal factors (*MTHFR* C677T polymorphism) and exogenous maternal factors (body mass index and dietary habits such as folate intake) on maternal LINE-1 methylation and on the occurrence of CHD in children with DS. Study showed that the *MTHFR* genotype/diet combination and BMI were significantly associated with LINE-1 methylation in mothers of children with DS/CHD$^+$ [5]. Recently, micro-RNA signatures discordant for CHD in monozygotic twins were observed [112].

4.3 DNA methylation in developmental exposure to the maternal environment and diet

It has been suggested that disease risk of long-term health outcomes may be in part determined by maternal (in utero effects of environmental exposures, toxins/nutrition) [21, 113] and paternal diet [114, 115].

Birth defects occur in 6–10% of babies born to mothers with pregestational diabetes, which is a significant health problem. It has been demonstrated that exposure to maternal diabetes during pregnancy changes gene expression levels in the mouse embryo, disrupting essential cellular activities [116], and could lead to disruption of crucial epithelial and mesenchymal cell interactions in developing kidney, leading to kidney and urinary tract malformation [117]. Underlying mechanisms are still unknown. There is a proposed lack of precision in the developmental program, which is essential for organogenesis induced by hyperglycemia effects on oxidative stress. That exposure to a diabetic intrauterine environment during pregnancy could be teratogenic by leading to defects like CAKUT in the fetus and associate with metabolic or cardiovascular diseases in later life [118–121].

Changes in maternal dietary FA can affect the DNA methylation patterns of offspring in mice [61]. The *agouti* mouse is a best-studied example [122]. Recently, in the human genome, loci were found to show differential methylation in response to season of birth that is similar to the *agouti* locus, but the identity of the causative agent for the changes in DNA methylation is unclear [123]. Recent study examined the prospective association between multivitamin supplementation during pregnancy and maternal plasma folate/vitamin B_{12} levels at birth and child's autism spectrum disorder (ASD) risk. Moderate (3–5 times/week) self-reported supplementation during pregnancy was associated with decreased risk of ASD, consistent with previous findings. But, extremely high maternal plasma folate and B_{12} levels at birth were associated with ASD risk. This study raises new questions about the impact of extremely elevated levels of plasma folate and B_{12} exposure in utero on early brain development [124]. However, study on postmortem cortical brain samples reveals that global DNA methylation was markedly enriched in ASD brains [125]. In some diseases, methylation mosaicism was found to be present. This is a common phenomenon in Fragile X syndrome (FXS). A decreased gene expression was found to be a main contributor to the cognitive impairment observed in the study of 12 FXS males with atypical mosaicism, seven of whom presented with ASD [126].

5. Epigenetic pattern transmission from parent to offspring: understanding disease inheritance

The heritability of epigenetic modifications, including histone modifications and DNA methylation, provides a memory of cell function and identity. Transmission of epigenetic information to subsequent generations may provide evolutionary mechanisms that impact on adaptation to changed environment. Defining the mechanisms that establish and regulate the transmission of epigenetic information from parent to offspring is critical for understanding disease heredity. Detection of modified methylation patterns is important in inappropriate imprinting of certain either maternal or paternal genes, which are "turned on" by epigenetic phenomenon that leads to diseases such as Angelman syndrome and Prader-Willi syndrome. Methylation patterns with detrimental effects on development have been established for disorders of methylation, by several groups of researchers [127, 128]. One of the developed blood tests (EpiSign) claims to diagnose 19 congenital diseases [129]. Also, it is important to establish the potential for epigenomic drugs that have an impact on the germline epigenome and subsequent offspring [130, 131]. Currently, the molecular pathways that regulate epigenetic information in the germline and its transmission to offspring are poorly understood. Recent study reveals a novel role for the histone-modifying complex, PRC2, in maternal intergenerational transmission of epigenetic effects on offspring, with important implications for understanding disease inheritance [115]. PRC2 is involved in the regulation of many fundamental biological processes and is especially essential for embryonic stem cells. However, how the formation and function of PRC2 are regulated is mostly unknown. Recent findings identify miR-323-3p as a new regulator for PRC2, providing a new approach for regulating PRC2 activity via microRNAs [132]. Specific epigenetic pattern was observed to be essential in the development of CHD and CAKUT. Impaired transcriptional profiles in individuals with CHDs [133] and CAKUT [134, 135] were shown to be affected by epigenetic regulators of gene expression, using bioinformatical analysis and integrated prediction algorithms [136]. The miRNA-145 expression was confirmed in infants with CHD that negatively regulates gene expression important for heart development [133]. The altered hsa-miR-144 expression was, for the first time, identified in CAKUT and could be connected with biological processes crucial for normal development of kidney and urinary tract [135]. Although the importance of mothers' health prior to conception and during pregnancy is now well accepted, recent data also implicate fathers' health/nutritional status (overnutrition, undernutrition, and other forms of stress) in contribution to the risk of metabolic disease and obesity in offspring. Epigenetic paternal inheritance of chronic disease provides novel opportunities for multigenerational disease prevention [137]. Germ cell-dependent mechanisms have recently been linked to these intergenerational effects. There is increasing evidence that disruptions in male germ cell epigenetic reprogramming are associated with offspring abnormalities. Adequate supply of methyl donors is required in the fetal period, which is the critical time of DNA methylation pattern acquisition for developing male germ cells. In addition, DNA methylation patterns continue to be remodeled postnatal during spermatogenesis. Previous studies have shown that lifetime (prenatal and postnatal) folic acid deficiency and high-dose supplementation can alter the DNA methylation in sperm [138]. Recent study examined the genome-wide DNA methylation patterns in placentas and embryos in correlation with maternal FA supplementation in the prevention of CAs associated with assisted reproductive technologies (ART). Results demonstrate dose-dependent and sex-specific effects of FA intake; moderate dose of FA supplements may be optimal in ART for both sexes [139]. Even more, recent data suggest that genome-wide DNA methylation in the

placentas from preterm infants could be associated with maternal socioeconomic status [140]. On the other hand, genomic information was identical in monozygotic twins, but they could be discordant for congenital renal agenesis which could be a consequence of epigenomic regulation of gene expression [141].

6. Future perspectives

CAs are complex traits with polygenic, epigenetic, and environmental components. Advances in human DNA methylation research and growing epigenetic data offer a new avenue for the translation of research to clinical applications. Current methylome analysis has been helpful in major human diseases revealing an epigenetic influence, but current approaches are inadequate for the translation of these advances to clinical diagnostics. There is a need to deal with big data in modern genomic medicine, so bioinformatics and applied mathematics are of a fundamental help in simulation studies and tests of methylome datasets. Signal detection theory and machine learning approaches applied on methylome datasets from ASD patients demonstrate high discriminatory power for the methylation signal induced by disease [142]. Even more, advanced machine learning analysis includes a combination of active learning and imbalanced class learning and deep learning to develop a more efficient feature selection process and for the generation and simultaneous computation of any genomic or biological dataset applied to medicine [143]. This approach demonstrates the feasibility in clinical diagnostics. Genetic risk scores (GRS) are widely used for risk prediction in complex diseases. Evidence is growing that methylation risk scores (MRS) may be constructed for multiple health purposes. MRS is defined as weighted sums of the individual's methylation markers' beta values of a preselected number of CpG sites and can be useful in interaction and mediation analyses, for environmental exposures as biomarker, and for prediction of individual risks of disease predisposition or treatment success [144]. As we know that methylation data is specific (for different tissues) and sensitive to confounding factor, e.g., by age or sex, adaption of current GRS approaches is complex and needs deep profiling in construction of such risk scores. The analysis of whole biomarker genomic and epigenomic regions and prediction of disease predisposition, course and therapy response by risk scores could in future suffice for a diagnostic and decreasing cost of patients' treatment.

7. Conclusion

The heritability of epigenetic modifications, including histone modifications and DNA methylation, provides a memory of cell function and identity. The dynamic reprogramming and other epigenetic patterns which could affect normal patterns of gene expression/genome stability during development could lead to an increased risk of CAs or complex diseases later in life. The sperm- and egg-specific DMT established during gametogenesis at imprint and non-imprint loci are susceptible to environmental factors. Embryogenesis may also present a window of opportunity for environmental influences on DNA methylation pattern. Changes in maternal dietary FA can affect the DNA methylation of offspring that could affect CA development. LINE-1 hypomethylation in the parental germline might also significantly affect genome stability during the fetal development. The *MTHFR* T carriers have lower enzyme activity, and dose of supplementary FA shows a positive relationship with DNA methylation patterns in humans. The lowest values of LINE-1 methylation, the surrogate marker for global DNA methylation,

were observed in mothers with MTHFR 677 CT+TT genotype and low dietary folate, suggesting the possibility of impaired DNA methylation causing maternally derived trisomy 21. Also, *MTHFR* genotype/diet and BMI combination influence LINE-1 methylation in mothers that could be a risk factor for DS/CHD$^+$ development in children. The studies discussed in this chapter provide new evidence to support nutrigenomic personalized FA treatment of mothers with risk genotype to prevent global DNA hypomethylation as potential underlying mechanism of CA development.

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