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REVIEW ARTICLE

Role of genetic and environmental factors in DNA methylation of lipid metabolism

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Abstract A number of recent studies revealed that DNA methylation plays a central role in the regulation of lipid metabolism. DNA methylation modifications are important regulators of transcriptional networks that do not affect the DNA sequence and can translate genetic variants and environmental factors into phenotypic traits. Therefore, elucidating the factors that underlie inter-individual DNA methylation variations gives us an opportunity to predict diseases and interfere with the establishment of aberrant DNA methylation early. In this review, we summarize the findings of DNA methylation-related studies focused on unravelling the potential role of genetic and environmental factors in DNA methylation and the regulatory effect of DNA methylation on gene expression in lipid metabolism.

Introduction

DNA methylation, the most well-known epigenetic modification, primarily occurs on a cytosine preceding a guanine (CpG dinucleotide). The DNA methylation mechanism in mammals is achieved by two components: DNA methyltransferases (DNMTs), which are responsible for setting up and maintaining DNA methylation patterns, and methyl CpG binding proteins (MBDs), which are responsible for recognizing methylation-related marks. Their joint action

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maintains the dynamic changes of DNA methylation over time.[1] Even though approximately 70%—80% of CpG sites are methylated in human somatic cells, DNA methylation levels are unevenly distributed throughout the genome with lower methylation levels of CpG sites in promoter regions and CpG islands (CGI) than in non-CGI regions.[2] DNA methylation is related to multiple biological processes, such as transcriptional regulation during embryonic development, gene imprinting, inactivation of the X-chromosome and regulation of gene expression.[3] In recent years, new technological advances have provided a mapping of DNA methylation patterns and enabled studies on the regulation of expression levels by DNA methylation regulates.[4] DNA methylation is involved in transcription mechanisms by directly regulating the binding of specific transcriptional factors and by indirectly modifying the recruitment of MBDs and related chromatin remodelling.[1] Given the relationship between DNA methylation and gene expression, it's convincing that aberrant DNA methylation patterns can respond to the effects of genetic variants and environmental factors and can exert their influence on altered phenotypes or diseases.[5]

This review systematically describes the interplay between genetic factors, environmental factors, DNA methylation and gene expression related to lipid metabolism. Characterizing the genetic and environmental control of methylation and its contributions to the regulation of gene expression in lipid metabolism may help us understand the underlying biological mechanism and identify new biomarkers and treatment targets for metabolic diseases.

Regulatory role of DNA methylation on gene expression in lipid metabolism

DNA methylation is a noteworthy and unique process that plays a functional role in transcriptional regulation without alteration of the basic genetic sequence. The mechanism may be due to the addition of a methyl group on the C5 position of cytosine, which changes the chromatin structure and alters the access of transcriptional factors to regulatory regions.[6]

Some studies have examined methylation levels of CpG sites in the promoter region of specific genes involved in lipid metabolism. Guay SP et al analysed leucocyte DNA methylation in the low-density lipoprotein receptor (*LPL*) promoter region in 98 untreated familial hypercholesterolaemia patients and showed that DNA methylation was positively correlated with high-density lipoprotein cholesterol (HDL-c) levels in blood. The result was validated in visceral adipose tissue (VAT) from 30 severely obese men. In addition, they showed that DNA methylation at *LPL* was negatively correlated with relative mRNA levels in VAT.[7] The complex association suggested that DNA methylation can functionally regulate gene expression. Another study conducted in 73 severely obese patients showed that DNA methylation of specific CpG sites in the leptin (*LEP*) promoter region was positively correlated with low-density lipoprotein cholesterol (LDL-c) levels and negatively associated with *LEP* mRNA levels in subcutaneous adipose tissues (SAT). After adjusting for DNA methylation, the association between LDL-c and *LEP* mRNA was no longer significant.[8] These results indicated that the association between gene expression and LDL-c levels was partly attributed to DNA methylation variability.

Additionally, global DNA methylation maps are available to identify novel CpG sites that are associated with related phenotypes. Irvin et al evaluated the DNA methylation of lipid-related genes in $CD4^+$ T-cells from 991 individuals on a genome-wide level. They found that methylation of one CpG upstream of a promoter region of carnitine palmitoyltransferase 1A (*CPT1A*) was strongly associated with TG and negatively correlated with *CPT1A* expression in both the discovery and replication populations. Furthermore, mediation analysis suggested that gene expression changes in *CPT1A* explained 13.5% of the observed association of *CPT1A* DNA methylation at the CpG site with TG. The authors used ENCODE data to find several transcription factor binding sites and enhanced transcription marked by increased signals of acetylation of lysine 27 of the H3 histone protein (H3K27Ac) near the highlighted CpG. This evidence proved that the region of interest was an active regulatory site, providing powerful proof of the involvement of DNA methylation in gene expression.[9] Later, the association of DNA methylation in this CpG site with TG was proven in another study. In this study, genome-wide DNA methylation patterns were determined in whole blood samples of 1776 subjects in the discovery cohort and further replicated in two population-based cohorts. They found that a CpG site located in ATP binding cassette subfamily G member 1 (*ABCG1)* was negatively associated with HDL-c. The identified associations were then explored through gene expression and functional studies. Gene expression analysis identified a negative association between methylation at *ABCG1* and mRNA levels, and functional analysis by electrophoretic mobility shift assay revealed the higher binding affinity of a protein complex for the unmethylated status of the highlighted CpG compared with the methylated status. These results indicated that transcription factor binding was one of the mechanisms that mediated the identified negative association between *ABCG1* methylation and *ABCG1* mRNA levels.[10]

These studies suggested that DNA methylation is associated with gene expression, and the interaction explains related phenotype changes, such as serum lipid traits. Notably, one challenge of DNA methylation analysis in blood samples is cell heterogeneity. This observation was supported by Liliane Pfeiffer et al, who showed varying expression patterns between different blood cell types. After adjustment for estimated blood cell types, the number of significant CpG sites that were associated with gene expression declined greatly.[10] In addition, a global DNA methylation analysis was performed in different cell types in blood of six health males, including mononuclear cells, granulocytes, and seven isolated cell populations ($CD4^+$ T cells, $CD8^+$ T cells, $CD56^+$ NK cells, $CD19^+$ B cells, $CD14^+$ monocytes, neutrophils, and eosinophils). They found that many CpG sites were differentially methylated among different cell types with B cells showing the most special methylation pattern and these methylation differences were clustered together to reflect the corresponding cell types according to their expected haematopoietic lineage.[11] Consistently, a study on the methodology of

quantifying the normally mixed composition of leukocytes showed that DNA methylation arrays could serve as surrogate measures of cell mixture distribution.[12] Thus, it is necessary to either analyse DNA methylation with adjustments or separately analyse DNA methylation in each cell type, which provides a better understanding of which cell type plays a functional role. Additionally, DNA methylation might have a tissue-specific impact on gene expression. As observed in the study by Guay SP et al, the association of *LPL* DNA methylation and relative mRNA levels existed in VAT, but not in blood or SAT.[7] Thus, DNA methylation studies in different tissues may be useful for assessing the functional role of DNA methylation and directly targeting to a disease or trait of interest. Moreover, aside from DNA methylation, additional epigenetic events, including histone modifications, transcription factor binding and small RNAs, also account for gene expression variations. Therefore, a complex analysis of multiple factors should be considered regarding transcriptional alterations.

Genetic effect on DNA methylation of genes involved in lipid metabolism

Alterations in DNA methylation affect transcription, but the source of methylation variation itself remains poorly understood. Genetic variants may be the potential functional mechanism underlying inter-individual variation in DNA methylation levels. The interactions between genetic variants and DNA methylation variation have been studied on a genome-wide scale in multiple human tissues and cells, such as adipose tissue, pancreatic Islets, brain, blood and lymphoblastoid cell lines.[13–18] Analysis of methylation quantitative trait loci (meQTL) showed that 28% of CpG sites were associated with a nearby SNPs and that the genetic variants controlling DNA methylation were predominantly located in *cis*.[19] Furthermore, an overlap of meQTLs and expression quantitative trait loci (eQTLs) was observed in lymphoblastoid cell lines, indicating that some genetic variants relate to both DNA methylation and gene expression variation.[18]

A meta-analysis of 7 cohorts aimed to explore the effect of genetic variants on DNA methylation. They found that the C allele of rs2246293 was associated with higher methylation in the promoter region of ATP binding cassette subfamily A member 1 (*ABCA1)* and corresponded to lower ABCA1 expression and lower circulating HDL-c levels.[20] Furthermore, a genome-wide DNA methylation analysis in 144 human liver samples showed that rs174537 was associated with DNA methylation of CpG sites located in a putative enhancer signature region in the fatty acid desaturase (*FADS*) gene cluster and corresponded to alterations in polyunsaturated fatty acid levels.[21] In addition, a genome-wide mQTL analysis conducted by Petr Volkov et al in human adipose tissue found some SNPs associated with DNA methylation. These mQTLs overlapped with previously reported SNPs that were related to lipid traits in (genome-wide association studies) GWAS, including one SNP located in cholesteryl ester transfer protein (*CETP)* that was associated with DNA methylation of this gene and serum HDL levels (Table 1).[13] In another study, the author performed genome-wide gene expression and DNA methylation analyses in blood samples and combined the previous GWAS study with TG; the results showed that 12 SNPs of IQ motif-containing J (*IQCJ)* and 26 SNPs of neurexophilin-1*(NXPH1)* were associated with DNA methylation of specific CpG sites. Moreover, two SNPs of *IQCJ* were related to different splicing, and one SNP in *NXPH1* was associated with gene expression levels.[22]

It is well established that the interaction of genetic and DNA methylation variation contributes to the development of many diseases.[23,24] These studies have collectively suggested that genetic variants can originally regulate DNA methylation of genes involved in lipid metabolism that are also associated with serum lipid levels. However, the casual relationship between DNA methylation and lipid traits is not clearly defined. Further studies are needed to determine the directionality of the association regarding causality. Mendelian randomization is likely an effective way to investigate the causal relationship between an exposure and outcome of interest by using genetic variants as instrumental variables. Simultaneously, it reduces the influence of cofounders, which inevitably exist in DNA methylation studies.[25] Of note, approximately 10% of eQTLs were found to be related to DNA methylation variation in lymphoblastoid cell lines from 77 HapMap Yoruba individuals.[18] This finding was consistent with the results from a gene-specific methylation analysis in human brain. However, no co-regulation of methylation and expression by the same genetic variants was found in another study. These results suggest that methylation variation accounts for only a small part of the genetic effect on gene expression variation.

Regulatory effect of lifestyle on DNA methylation related to lipid metabolism

DNA methylation modifications due to genetic variants are considered to be a potential mechanism underlying expression alterations of genes involved in lipid metabolism. Importantly, DNA methylation is plastic in response to environmental factors and accounts for alterations in gene expression with or without associated phenotypic consequences.[26,27] High-fat diets (HFD) and sedentary lifestyles are the leading causes of multiple metabolic diseases. Extensive reprogramming of the DNA methylation patterns by lifestyle modifications was observed in many studies.[28–30]

Consumption of energy-enriched foods that are high in fat is associated with a risk of metabolic diseases. Peroxisome proliferator-activated receptor-γ coactivator-1a (*PGC1a)*, a transcriptional coactivator, is involved in lipid oxidation, mitochondrial function and the remodelling of muscle tissue.[31] Recent reports showed that PGC1a was differentially methylated in response to a 5-day high-fat diet in SAT and skeletal muscle in a birth-weight specific manner.[32,33] Later, the same group conducted a genome-wide DNA methylation analysis in skeletal muscle and SAT separately in healthy young men.[34,35] In skeletal muscle, HFD introduced widespread DNA methylation changes in healthy young men, whereas the related methylation changes in SAT were less extensive. Some of the differentially methylated CpG sites were located in genes involved

Table 1 Significant associations of single-nucleotide polymorphisms (SNPs) with DNA methylation of genes involved in lipid metabolism.

Study	Gene	SNP	CpG site	CpG site positions	Technique
Yiyi Ma et al	APOE	rs405509	cg04406254	CHR 19: 45407945, TSS1500	Infinium Human
	ABCA1	rs2246293	cg14019050	CHR 9: 107690770, TSS1500	Methylation 450K BeadChip
Timothy D et al		rs174537	cg27386326	CHR 11: 61587980, Intergenic region	Infinium Human
	FADS1	rs174537	cg16213375	CHR 11: 61584727, TSS200	Methylation 450K BeadChip
			cg10515671	CHR 11: 61585899, TSS1500	
	FADS2	rs174537	cg19610905	CHR 11: 61596333, Gene body	
Petr Volkov1 et al	CETP	rs1800775	cg02254551	CHR 16: 56998544, Gene body	Infinium Human
		rs3764261	cg26916607	CHR 16: 56994770, TSS1500	Methylation 450K BeadChip
	APOA5	rs2266788	cg12556569	CHR 11: 116664039, TSS1500	
	FADS2	rs1535	cg19610905	CHR 11: 61596333, Gene body	
	ACADS	rs2066938	cg21721566	CHR 12: 121163144, TSS1500	Infinium Human
			cg24768164	CHR 12: 121163261, TSS1500	Methylation 450K BeadChip
Bastien Vallée Marcotte et al	IQCJ	rs2044704	cg16975599	CHR 3: 158962761, Gene body	Infinium Human
	NXPH1	rs2349780	cg06056929	CHR 7: 8476128, Gene body	Methylation 450K BeadChip

The Infinium Human Methylation 450 BeadChip was used to estimate DNA methylation level in these four studies. The CpG sites mean the locus identified from the Illumina CG database. The CpG site positions refer to the genomic location in relation to the nearest gene including genomic regions TSS1500 and TSS200 (1500—201 and 200—0 bases upstream of transcription start site (TSS), separately), gene body and intergenic regions according to genome build 37. APOE, apolipoprotein E; ABCA1, ATPbinding cassette subfamily A member 1; FADS1 and FADS2, fatty acid desaturase 1, fatty acid desaturase 2; CETP, cholesteryl ester transfer protein; APOA5, apolipoprotein A5; ACADS, encoding acyl-CoA dehydrogenase; IQCJ, IQ motif-containing J; NXPH1, neurexophilin-1. CHR, chromosome.

in lipid metabolism. However, few studies demonstrated a significant correlation between DNA methylation and mRNA expression of relevant genes. The specific mechanism was not clearly defined. However, one study in SAT proved the correlation between DNA methylation and mRNA expression after insulin infusion, but not in the fasting state. Therefore, the correlation may only be masked when metabolic challenges induce a functional consequence mediated by DNA methylation.[32] Based on the observation that HFD-induced methylation changes were slowly reversed after the intervention of the control diet, another explanation may be that a 5-day high-fat overfeeding induces methylation changes but is too short for the induction of changes in gene expression levels. Thus, further studies are needed to explore whether persistent HFD may induce permanent alterations in DNA methylation and thereby changes in transcriptional consequences.

Physical activity is an effective therapeutic strategy for improving lipid metabolism and multiple metabolic diseases. Changes in DNA methylation levels are suggested to be one of the underlying mechanisms conveying the beneficial effects of exercise.[36—38] In adipose tissue from healthy, middle aged men, six months of exercise induced a widespread increase in DNA methylation. In this study, the authors also observed overlapping changes in DNA methylation and mRNA expression of relevant genes, including histone deacetylase 4 (*HDAC4)* and nuclear receptor corepressor 2 (*NCOR2)*. The two genes were selected for functional validation in adipocytes, and the results showed that silencing of *HDAC4* and *NCOR2* increased lipogenesis.[38] In skeletal muscle of healthy sedentary men and women, acute exercise induced a general global decrease in DNA methylation.[37] In the two studies, we observe that exercise induces opposite changes to DNA methylation levels. An explanation may be provided by differences in the type of exercise, tissues and participants. Moreover, *PGC1a*, a key metabolic regulator, was identified to be differentially methylated in response to exercise. An acute bout of exercise-induced hypomethylation of the *PGC1a* promoter region was observed in a dose-dependent manner along with a corresponding increase in mRNA expression levels in skeletal muscle.[37] Additionally, a study confirmed the effect of maternal exercise on *PGC1a* methylation in the skeletal muscle of mouse offspring.[39] It is well known that parent-offspring transmission is an important determinant of offspring health. Thus, this study provides evidence on the effect of maternal exercise intervention in preventing metabolic dysregulation in offspring. Collectively, these findings showed exercise-induced DNA methylation changes in multiple genes in skeletal muscle and adipose tissue, which are important tissues in energy metabolism. The regulation of these genes is tissue-specific in response to exercise intervention. However, it is unclear whether different types of exercise intervention have different influences on methylation changes.

In addition, smoking and drinking-associated morbidity and mortality is also a challenge for public health systems. One of the potentially modifiable mechanisms could be DNA methylation. Many studies have demonstrated the effect of alcohol and cigarette consumption on global DNA methylation. Breitling LP et al performed methylation analysis in peripheral-blood DNA of 177 current smokers, former smokers and those who had never smoked. They found that the methylation level of one CpG site located in coagulation factor II receptor-like 3 (*F2RL3*) was lower in smoker.[40] Methylation of this gene was further proved to mediate the detrimental impact of cigarette consumption and to be strongly associated with coronary heart disease.[41,42] In addition, a genome-wide DNA methylation study conducted in peripheral blood mononuclear cells from African

American women demonstrated that methylation level of two CpG sites in Aryl Hydrocarbon Receptor Repressor (*AHRR*) was significantly different between smokers and non-smokers.[43] Furthermore, by using of a genome-wide approach, Robert A Philibert et al showed that short-term alcohol treatment induced significant changes in DNA methylation and the observed effect tended to diminish with abstinence.[44] However, few studies have focused on the effect of smoking or alcohol consumption on DNA methylation of genes involved in lipid metabolism. Thus, further study is needed to clarify whether DNA methylation acts as a mediator in the association of smoking and drinking with lipid metabolism.

These studies uncovered that DNA methylation links lifestyle to transcriptional and functional consequences in lipid metabolism, thereby providing powerful evidence for the beneficial effect of a healthy lifestyle in preventing metabolic diseases.

Regulatory effect of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) and vitamin intake on DNA methylation in lipid metabolism

Emerging evidence showed that n-3 LCPUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have the beneficial effect of delaying the development of various metabolic diseases. The relationship between n-3 LCPUFA intake and DNA methylation patterns is well documented in multiple tissues, including blood mononuclear cells and adipose tissue.[33,45,46]

Some studies have examined the effects of selected genes on DNA methylation. One study investigated the effects of an 8-week intake of n-3 LCPUFA on the DNA methylation profile of selected genes in 12 overweight women. The expression of these genes was previously shown to be modified by n-3 LCPUFA in peripheral blood mononuclear cells. However, the authors showed that the DNA methylation levels of these genes were slightly regulated by the n-3 LCPUFA intervention. The results suggested that the observed association of n-3 LCPUFA intake and gene expression was not mediated by DNA methylation.[47] In contrast, another study observed significant changes in DNA methylation levels after fatty acid intervention. The study aimed at exploring the influence of n-3 LCPUFA and olive oil (OO) intake on the DNA methylation levels of selected genes, including FADS1, FADS2, ELOVL5 (elongase) and ELOVL2, in 29 patients with renal disease. These genes encode desaturase and elongase and are involved in the synthesis of LCPUFAs, which were shown to be associated with an improvement of lipid profiles and cardiovascular disease. They showed that both n-3 LCPUFA and OO induced significant changes in DNA methylation levels of specific CpG sites. In addition, the degree of changes in individual CpG loci were different with the supplement of n-3 LCPUFA and OO.[48] The two studies of selected genes suggested that only parts of genes that were previously demonstrated to be associated with n-3 LCPUFA intervention might mediate the effect of n-3 LCPUFA on DNA methylation. Therefore, a global DNA methylation profile is necessary for identifying DNA methylation changes in response to n-3 LCPUFA intake. A randomized controlled trial investigated the effect of feeding 31 healthy normal-weight individuals either PUFA or saturated fatty acids (SFA) for 7 weeks on the genome-wide DNA methylation levels in human subcutaneous adipose tissue. The global degree of DNA methylation levels increased with the intervention of both PUFA and SFA. In addition, 4875 CpG sites were differentially modified by PUFA and SFA supplements. They observed that 1797 genes were differentially induced by PUFA, whereas 125 genes were differentially induced by SFA. The results further validated the point that dietary components exert distinct effects on DNA methylation levels.[49] Moreover, based on the notion that n-3 LCPUFA can reduce circulating TG and raise HDL-c, one study further explored to what extent the regulation of n-3 LCPUFA on lipid traits is achieved by DNA methylation. In this study, they mapped the genome-wide DNA methylation profiles of blood leukocytes from 36 overweight and obese individuals and showed that n-3 LCPUFA supplementation induced the differential methylation of 308 CpG sites in 231 genes. Among these CpG sites, two CpG sites within insulin-like growth factor binding protein 5 (*IGFBP5*) and AKT serine/threonine kinase 3 (*AKT3*) were separately associated with changes in TG and TC/HDL-c. However, no significant association between changes in DNA methylation and gene expression levels was observed for these two genes, suggesting that the observed correlations of changes in DNA methylation levels with altered lipid levels may not be associated with changes in gene expression levels.[50]

These studies provided evidence of the association between n-3 LCPUFA intake and DNA methylation of biologically relevant genes. They also suggested that changes in DNA methylation specifically respond to different types of dietary fatty acids. The specific mechanism of how n-3 LCPUFA intake impacts DNA methylation levels may involve one-carbon metabolism, in which methyl groups are provided via S-adenosyl methionine (SAM).

In addition to n-3 LCPUFA, Vitamin B12 and folic acid (Vitamin B9) are also important factors that can transfer a methyl group to DNA via SAM and regulate the expression of many genes. Evidence showed that methionine limitations might affect lipid metabolism via limited methylation capacity.[51] The impact of maternal vitamin B12 and folic acid intake on foetal lipid metabolism via DNA methylation was demonstrated.[52] One study analysed the epigenetic effects of a methyl donor-deficient diet or a standard diet during gestation and lactation in the liver of rat offspring. They showed that methyl donor-deficiency diet-induced changes in DNA methylation and gene expression levels involved multiple pathways, including lipid metabolism.[53] In addition, high levels of folate and vitamin B12 supplements during pregnancy may have induced DNA methylation changes and subsequently adiposity in mouse offspring.[54] Evidence showed that DNA is more sensitive to epigenetic reaction-related enzymes during cell division.[55] Thus, early changes in maternal dietary nutrition can induce alterations in the DNA methylation of offspring. Although the vitamin B12 and folic acid intake during the perinatal period is important for the metabolic state, the supplement is also crucial in adults. Some studies have explored the effect of vitamin B12 and folic acid intake in adults on the methylation of genes

involved in lipid metabolism. A population-based intervention study was conducted by Krista S. Crider et al to investigate the effect of a 6-month intake of folic acid supplementation on DNA methylation in women of reproductive age. The authors observed significant changes in DNA methylation levels after folic acid intervention in DNA extracted from coagulated blood.[56] These studies revealed an important role of vitamin B12 and folic acid intake on DNA methylation. However, the underlying mechanism of the biological function of vitamin B12 and folic acid intake needs to be explored.

Conclusions and perspectives

DNA methylation variation has identified key genes in lipid metabolism that contribute to the variability of DNA methylation levels. Genetic variants and environmental factors separately play important roles in determining stable and dynamic patterns of DNA methylation. DNA methylation can serve as an epigenetic proxy for genetic variants, which thereby can act as a biomarker by predicting susceptibility to metabolic diseases and can regulate the transmission of genetic information to the related phenotype by interfering with DNA methylation. Additionally, DNA methylation differences likely mirror the effect of environmental factors, providing evidence on the effects of exercise and nutritional and medical interventions on prevention in individuals with a high risk of metabolic diseases.

DNA methylation is a powerful tool for understanding the underlying biological mechanisms of diseases or other phenotypes. However, there are some limitations to DNA methylation analysis. First, DNA methylation is dynamic across a lifetime and studies of DNA methylation are inevitably influenced by other confounding factors.[57] Thus, it is necessary to evaluate DNA methylation with adjustments for confounders, such as age, sex, BMI, cell type proportion, smoking, alcohol consumption and medication.[10] Moreover, DNA methylation analysis of specific tissues associated with related diseases or phenotypes has high fidelity in reflecting the disease state or predicting the disease risk. Second, there are multiple inconsistencies in the potential causal relationships between DNA methylation and related phenotypes. Further work is required to help make causal inferences about the relationships between DNA methylation, gene expression and phenotypes. Third, differences in DNA methylation assays may influence the evaluation of DNA methylation levels. Therefore, technical replicates are suggested to estimate intrinsic variability and obtain more practical results.

Conflicts of interest

The authors have declared that no competing interests exist.

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