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Lipid Phenotypes and DNA Methylation: a Review of the Literature

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Abstract

Purpose of Review Epigenetic modifications via DNA methylation have previously been linked to blood lipid levels, dyslipidemias, and atherosclerosis. The purpose of this review is to discuss current literature on the role of DNA methylation on lipid traits and their associated pathologies.

Recent Findings Candidate gene and epigenome-wide approaches have identified differential methylation of genes associated with lipid traits (particularly *CPT1A*, *ABCG1*, *SREBF1*), and novel approaches are being implemented to further characterize these relationships. Moreover, studies on environmental factors have shown that methylation variations at lipid-related genes are associated with diet and pollution exposure.

Summary Further investigation is needed to elucidate the directionality of the associations between the environment, lipid traits, and epigenome. Future studies should also seek to increase the diversity of cohorts, as European and Asian ancestry populations are the predominant study populations in the current literature.

Keywords Lipids · DNA methylation · Dyslipidemia · Atherosclerosis · Epigenetics

Introduction

Lipids are a class of biomolecules that include fats, sterols, phospholipids, and more. Their primary function in humans is to store energy and provide structural support to the cell membrane [1]. However, dysfunction of lipid metabolism, storage, or clearance may result in abnormal blood lipid levels, i.e., dyslipidemia [2]. An independent risk factor for cardiovascular disease, dyslipidemias, arises through both monogenic mutations (e.g., low density lipoprotein receptor (*LDLR*) in familial hypercholesterolemia) and polygenic influences, such as single nucleotide polymorphisms (SNPs) [3]. This is supported by the high heritability of plasma lipids: estimates for high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), and total cholesterol (TC) range from 40 to 60% [4]. Yet, even with the incorporation of rare variants, known genetic variants account for 10 to 25% of the total variation in lipid levels [5, 6]. This suggests that complex interactions between the genome and environment remain unaccounted for in the pathogenesis of dyslipidemia. Other genomic modifications (e.g., epigenomics) have been theorized to contribute to the "missing heritability" of lipids.

DNA methylation is the most common form of epigenetic modification and involves the addition of methyl ($-CH_3$) groups to cytosine-phosphate-guanine (CpG) sites of genes. Generally, increased methylation in gene promoters—regions of DNA that regulate gene expression via activation of transcription—is associated with decreased gene expression, and decreased methylation is associated with increased gene expression. The relationship between DNA methylation and lipids, as well as pathologies associated with abnormal

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lipid levels (dyslipidemia, atherosclerosis, etc.), has been extensively studied. Specifically, candidate gene and epigenome-wide association studies (EWAS) have identified differential methylation of genes in connection with lipid phenotypes. However, some questions remain, such as the directionality of the relationship between methylation and lipid levels, as well as whether the observed methylation variations in blood (the predominant tissue sample in these analyses) reflect gene regulation in target tissues. Furthermore, findings are mixed on the effect of environmental factors on lipids via DNA methylation. In this review, we will explore novel methodological approaches to explicate the relationship between lipids and methylation; evaluate the current literature on methylation variations at genes that have been widely replicated in association with plasma lipids; and highlight environmental exposures that have been linked to both methylation changes and lipid traits.

Methodological Approaches

EWAS

Early studies of DNA methylation and lipids predominantly applied candidate gene and EWAS approaches. Candidate gene studies test for associations between methylation and preselected genes of interest. In recent years, they have been used primarily in case–control studies and smaller cohorts ($n < 100$), and, for the purpose of this review, we will not discuss those smaller studies. Moreover, many EWAS—which use a hypothesis-free approach to identify associations between methylation of individual CpG sites and the trait of interest—since 2016 have included some form of validation (split sample approach or external validation). Meta-analyses are also becoming an increasingly useful tool to increase sample size and statistical power to detect significant CpG sites across cohorts. To date, most EWAS of lipids have been cross-sectional in design and conducted predominantly in populations of European or Asian ancestry. These studies have not only evaluated methylation variations in association with lipid levels directly [7–10], but also lipidomic profiles [11], plasma lipoprotein A [12], and pathologies such as atherosclerotic stroke [13]. However, a limitation of this approach is that it considers the effects of individual CpG sites, whereas multiple CpGs may be present in a single promoter region. Studies that evaluate the combined effect of methylation variations of CpG sites, e.g., differentially methylated regions (DMRs), are lacking.

Multi-"omics" Approaches

Methylation analyses have begun to integrate other "-omics" data to evaluate the joint effects of both the genome and epigenome on gene expression. While these studies are not as abundant as EWAS, they have been increasing in frequency. For example, some investigators have begun to incorporate genome-wide association study (GWAS) data into their methylation analyses. In a cohort of ~ 700 older African American adults, Wright et al. evaluated the association between serum lipids and DNA methylation sites that were proximal to single nucleotide polymorphisms (SNPs) previously linked to lipid traits [14]. Using a methylation-quantitative trait loci (meQTL) approach, Bandesh et al. identified functional variants in a cohort of ~ 230 Indian adults that were associated with methylation changes in the same genes with those significant variants [15]. In a GWAS meta-analysis, Ghanbari et al. explored associations between long non-coding RNAs (lncRNAs) and cardiometabolic disorders, in which they found that the methylation level of cg17371580 (located in the promoter of *LOC157273*) was associated with HDL [16]. The lncRNA gene, *LOC157273*, is an effector transcript located near *PPP1R3B*, which has also been linked to LDL and coronary artery disease [16–18].

In an expression-quantitative trait loci (eQTL) approach, Love-Gregory et al. identified lipid-associated SNPs located near the cluster of differentiation 36 (*CD36*) gene in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study. The association between those SNPs, CpG sites in the *CD36* promoter region, and *CD36* expression in adipose tissue from the Multiple Tissue Human Expression Resource (MuTHER) was then evaluated [19]. The investigators determined that both SNPs and methylation changes independently influence *CD36* expression. CD36 facilitates fatty acid uptake by the cell, and in previous studies, *CD36* variants have been shown to influence fasting lipid levels and risk for metabolic syndrome [20, 21]. Furthermore, Tremblay et al. applied a weighted gene correlation network analysis in a cohort of 16 families ($n = 48$) and identified several genes that were associated with HDL, LDL, and TC, as well as ApoB100 [22].

Tissue-Specific Methylation

While most human methylation studies have performed analyses from whole blood, a few have begun to explore tissue-specific methylation to further evaluate the relationship between DNA methylation and lipid-associated pathologies. In larger cohort studies, these tissues tend to be used for more targeted, functional analyses after identification of significant CpG sites from genome-wide analyses. For example, Pfeiffer et al. used skin ($n \sim 400$) and adipose ($n \sim 650$) tissue samples from the MuTHER cohort to evaluate the relationship between CpG sites that were significant in an EWAS of whole blood and gene expression [23]. Similar to the results from blood, increased methylation at sterol

regulatory element binding transcription factor 1 (*SREBF1)* was associated with elevated TG in both adipose and skin. Increased methylation at adenosine triphosphate (ATP) binding cassette subfamily G member 1 (*ABCG1)* was only associated with elevated TG in adipose. In another analysis, in subcutaneous fat from the TwinsUK registry (which has some overlap with MuTHER), cg20544516 methylation was negatively associated with *SREBF1* expression [24••]. These findings are comparable to EWAS in whole blood, which have shown increased methylation of this CpG site in association with elevated lipid levels [7, 24••]. Overall, the concordance between methylation analyses of lipid traits in whole blood and those in other tissues, particularly adipose, suggest that blood-based methylation studies are reflecting functionally important variations in DNA methylation at the tissue level.

While in larger cohort studies, tissue-specific methylation analyses tend to be applied for validation and/or secondary analyses, some smaller studies are using this approach for primary investigation. Adipose tissue samples collected from ~ 200 Canadian adults were analyzed by methylC-capture sequencing (MCC-Seq) to fine-map EWAS signals [25]. Investigators identified and externally validated adipose tissue regulatory regions that were associated with HDL and TG. Moreover, Lacey et al. identified tissue-specific differentially methylated regions (DMRs) in atherosclerosis using smooth muscle cells (SMCs) collected from patient aortas ($n = 3$); they found that methylation changes in aorta SMCs may downregulate enhancers to facilitate a pro-atherosclerotic phenotype [26]. The same investigators also identified tissue-specific regulation of *ANGTP* in atherosclerosis, in addition to methylation changes in enhancers regions, thus contributing to *ANGPT* expression [27]. Furthermore, Wang et al. observed that decreased methylation at genes in atherosclerotic right coronary artery tissue (compared to the great saphenous vein) were associated with pro-inflammatory pathways (e.g., cytokine receptor interactions); genes with increased methylation were associated with fat digestion and absorption pathways [28]. Tristán-Flores et al. characterized a differential methylation motif in human atheromas that was associated with Alu methylation, which is a hallmark of atherosclerosis [29]. Most studies of methylation and lipids are still based on whole blood samples, but some are beginning to evaluate methylation variations in vascular and adipose tissues with respect to lipids.

Finally, other approaches to evaluating DNA methylation and lipids are broadening from discovery and validation of individual genes or CpG sites of interest. More recent studies have included pathway [30•] and gene network analyses [22], indicating that researchers may be shifting from identifying methylation within individual genes to exploring how these modifications affect larger systems (e.g., regulatory pathways, gene networks, etc.) in disease. Methods are also being developed to employ epigenetic data as potential biomarkers. For example, Irvin et al. observed that epigenetic age acceleration (estimated from epigenetic clock algorithms) was positively associated with inflammatory markers and TG and negatively associated with HDL in the GOLDN study [31]. More studies, however, are needed before these tools are clinically translatable. Overall, the methods for understanding DNA methylation in the context of lipids are vast, as the field is shifting from candidate gene and EWAS-based approaches to more integrated "omic" analytical methods.

Widely Replicated Genes

CPT1A

Carnitine palmitoyltransferase 1A (*CPT1A*) codes for a key enzyme that initiates long-chain fatty acid beta-oxidation by the mitochondria, thus playing an important role in lipid metabolism. Multiple cross-sectional EWAS in whole blood have identified methylation variations of CpG sites annotated to *CPT1A* in association with blood lipid levels. In a population-based cohort study of ~ 1500 Dutch adults aged 45 years and older, Braun et al. identified differential methylation of two CpG sites (cg00574958 and cg17058475) in association with TG levels [7]. Methylation of these CpG sites were inversely associated with TG and very low-density lipoprotein (VLDL) cholesterol [32, 33]. GOLDN investigators also found that methylation of cg00574958, specifically, explained ~ 12% of the variation in TG [32]. Further study showed that decreased methylation of cg00574958 was linked to elevated plasma adiponectin levels [8]. Adiponectin is a hormone primarily secreted from adipose tissue, and it plays multiple roles in fatty acid oxidation, insulin resistance, and atherosclerosis [34]. This relationship not only remained significant after accounting for body mass index (BMI) and cigarette smoking, but also was replicated in a cohort of Amish adults ($n \sim 500$), as well as white, but not black, adults in the Bogalusa Heart Study ($n \sim 850$).

Furthermore, *CPT1A* methylation (specifically cg00574958) has been linked to other cardiometabolic traits and pathologies: hypertriglyceridemic waist phenotype, a potential marker of type 2 diabetes (T2D) [35]; familial hypercholesterolemia [36]; BMI [37]; adiposity [38]; carbohydrate and fat intake [33]; and metabolic syndrome [39]. A longitudinal study in rats suggested that high fat diet may increase *CPT1A* expression in blood [40], but human studies are lacking. Considering these findings, some investigators have sought to elucidate the causal relationship between *CPT1A* methylation and lipid traits through Mendelian randomization analyses. While these studies are not yet abundant, the current findings suggest that the lipid levels are

causal for methylation variations rather than methylation being causal for the lipid trait variation. Sayols-Baixeras et al. identified causal effects of fasting TG levels on the methylation of CpG sites annotated to *CPT1A* in a cohort of ~ 1000 adults [41]. Similarly, Dekkers et al. observed causal effects of TG on the same CpG site (cg00574958) in the BIOS Consortium (n ~ 2000) [42]. Moreover, a metabolomics study (n ~ 360) identified metabolite levels as causal on methylation of multiple CpG loci, including those annotated to *CPT1A* [43]. These findings suggest that *CPT1A* methylation plays an important role in cardiometabolic diseases but that the mechanisms are complicated and could be environmental.

ABCG1

ABCG1 codes for a protein involved in cholesterol transport in macrophages and lipid homeostasis. *ABCG1* methylation has previously been linked to T2D and glycemic traits, including as a potential mediator between statin use and T2D risk [44, 45]. EWAS have identified increased methylation at CpG sites annotated to *ABCG1* in association with elevated TG and lower HDL levels: cg06500161 and cg27243685 [7, 23, 24••]. Other studies have evaluated associations between these CpG sites and hypertriglyceridemic waist [35], metabolic syndrome [46], prior myocardial infarction [23], insulin resistance [47•], and adiposity [48]. Additionally, an EWAS of ~ 650 German adults in a population-based study (KORA F4) showed that methylation of cg06500161 was inversely associated with *ABCG1* expression in whole blood; this association was marginally significant after correction for multiple comparisons [24••]. These findings were supported by an earlier study in the KORA F4 cohort also reporting the inverse association between *ABCG1* methylation and corresponding mRNA transcripts as well as HDL levels; the investigators further identified a positive association between *ABCG1* mRNA and HDL levels, suggesting that the relationship between *ABCG1* methylation and HDL may be mediated by *ABCG1* expression [23]. Another study showed that methylation at cg07397296 partially mediated the relationship between in utero famine exposure and adult TG levels, and these methylation variations were associated with gene expression in an external dataset [49]. Overall, the directional relationship between *ABCG1* methylation and lipids has been difficult to assess from cross-sectional research, although a Mendelian randomization analysis showed a causal effect of HDL on methylation of *ABCG1* CpG sites [42]. Candidate gene and case–control studies have also linked increased methylation of *ABCG1* to atherosclerotic markers and elevated LDL [30•, 50]. In sum, differential methylation of *ABCG1* has been widely associated with lipids and related traits. Still, further analyses are needed to characterize the relationship between gene expression, environmental factors, methylation, and lipid levels.

SREBF1

SREBF1 binds a motif in the promoter region of the LDL receptor gene (*LDLR*) to activate its transcription for cholesterol metabolism. EWAS in whole blood have repeatedly found that increased methylation at cg11024682 and cg20544516 (located in the promoter region of *SREBF1*) is associated with a worsening lipid profile. Increased methylation of cg11024682 was also linked to elevated TG levels and postprandial lipemia in GOLDN [7, 33], as well as decreased HDL levels in the Registre Gironí del Cor (REGICOR), Framingham Offspring Study, and GOLDN cohorts (n ~ 3300) [51]. Moreover, a meta-analysis of European and Indian adults (n ~ 5500) showed that cg11024682 and cg20544516 methylation was positively associated with VLDL and TG levels [24••]. In secondary analyses (n ~ 1700), Gomez-Alonzo et al. also found that cg20544516 methylation was inversely related to expression of *SREBF1* cis-transcripts in subcutaneous fat. These findings are consistent with previous analyses showing that differential methylation of cg11024682 and cg20544516 in relation to lipid traits persist in both whole blood and adipose tissue. In a cohort of ~ 1800 adults, not only did methylation of these CpG sites in blood each explain ~ 3% of the variation in TG level, but they were differentially methylated in skin and adipose tissue samples from an external cohort [23]. Like *CPT1A* and *ABCG1*, Mendelian randomization has showed a causal effect of TG levels on methylation of this gene [42]. Moreover, increased methylation at *SREBF1* has been associated with central adiposity [52]; BMI [47•, 53–55]; childhood and adult obesity [30•, 56]; and T2D [57, 58]. These findings have been replicated in diverse cohorts, increasing their generalizability. Given the relationship between these CpG sites and several traits, *SREBF1* methylation may serve as a potential "multipurpose" biomarker for cardiometabolic dysfunction, not exclusive to lipids.

Overall, candidate gene and EWAS analyses have identified and repeatedly validated that *CPT1A*, *ABCG1*, and *SREBF1* are targets of DNA methylation in lipid metabolism and associated disease. Furthermore, Pfeiffer et al. have demonstrated how these genes are interrelated to regulate cholesterol homeostasis and fatty acid metabolism: *MIR33a/b* is co-transcribed with *SREBF1*, and the intronic miRNA acts as a negative regulator of *ABCG1* and *CPT1A* [23]. While current findings suggest that the relationships between lipid traits and methylation of these genes in blood are concordant with those in adipose tissue, more studies are needed to evaluate how these methylation variations affect gene transcript levels in target tissue. Other genes (e.g., *DHCR24*, *ABCA1*) may also be of interest, but they have

not been validated as extensively as those discussed above. More studies are needed to fully describe the directionality of the environment, methylation, and lipid traits.

Environmental Considerations

While Mendelian randomization studies of methylation and lipids are limited, current analyses suggest that methylation changes are the consequence of lipid traits rather than the cause, supporting an important role for exposures which induce methylation changes [42]. Prospective epigenetic studies of environmental factors also consistently show that external exposures may induce methylation changes relevant to lipids [59] (Fig. 1). Importantly, environmental epigenetic study findings have shown overlap with putative metabolic and lipid-related pathways, but the findings are mostly independent of previous lipid EWAS discoveries discussed above. These analyses have primarily focused in the areas of pollution and diet.

Pollution

Air pollution is a risk factor for exposure to particulate matter: hazardous, microscopic particles that are suspended in the atmosphere [60]. An EWAS meta-analysis in the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities (ARIC) studies identified significant methylation changes in association with particulate matter exposure ($PM_{2.5–10}$, PM_{10}), including at a CpG site annotated to miR128-2 [61]. This miRNA has been posited as an inhibitor of *ABCG1* [62], and its methylation could result in *ABCG1* upregulation. However, as previously discussed, *ABCG1* silencing (via increased methylation) has been associated with elevated lipid levels in EWAS studies. Studies are lacking on the association between miR128-2 and lipids, but these findings suggest that there may be complex and potentially multidirectional relationships between pollution, methylation, and lipids. Furthermore, in a randomized crossover study of healthy young adults in China ($n = 36$), investigators observed increased methylation in the promoter regions of additional sex comb-like 2 (*ASXL2*) and lamin A/C (*LMNA*) following short-term, high exposure to particulate matter ($PM_{2.5}$) [63]. *ASXL2* encodes a transcriptional regulator that facilitates lipid homeostasis via the PPAR-γ pathway [64]. *LMNA* encodes lamins A and C, structural nuclear proteins that contribute to lipid metabolism and storage. Mutations in *LMNA* can cause laminopathies, of which hyperlipidemia and atherosclerosis are clinical presentations [65]. More prospective studies in larger cohorts are needed, but preliminary analyses suggest that, at the very least, there is a connection between exposure to particulate matter and lipid-related genes via DNA methylation.

Other studies have explored the role of endocrine disrupting chemicals (EDCs), such as phthalates and parabens, in methylation changes. Among a cohort of Dutch adults, an EWAS identified differentially methylated CpG sites related to urinary concentration of EDCs. Multiple CpG sites were annotated to genes that are functionally related to TG and HDL levels [66]. Another EDC, di(2-ethylhexyl)phthalate (DEHP), is added to plastics to make them flexible, and chronic DEHP exposure may cause adverse cardiovascular

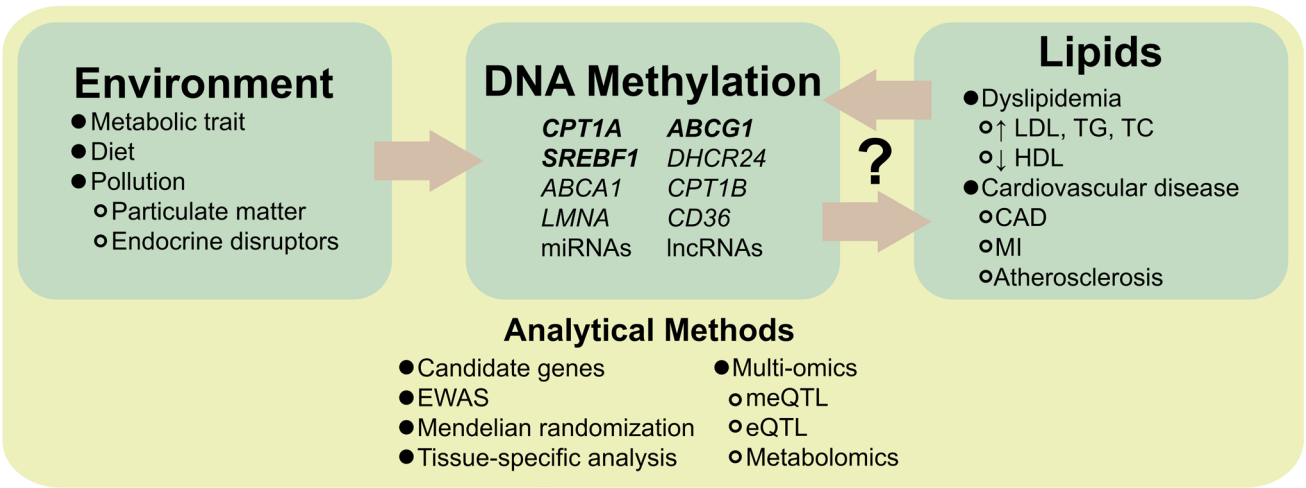

Fig. 1 The interrelation of the environment, epigenome, and lipids are not fully understood. Environmental factors may induce methylation changes at genes that are associated with lipid traits. Conversely, Mendelian randomization studies suggest that lipid traits are causal for methylation. Further studies (via a diverse set of analytical methods) are needed clarify the directionality of these relationships. Abbreviations: microRNA (miRNA); long non-coding RNA (lncRNA); low-density lipoprotein cholesterol (LDL); triglycerides (TG); total cholesterol (TC); high-density lipoprotein cholesterol (HDL); coronary artery disease (CAD); myocardial infarction (MI); epigenome-wide association study (EWAS); methylation quantitative trait loci (meQTL); expression quantitative trait loci (eQTL)

effects [67, 68]. In both animal models and epidemiologic studies, DEHP exposure was associated with global hypermethylation, elevated cholesterol levels, and carotid intima-media thickness (CIMT, a marker of subclinical atherosclerosis) [69, 70].

Heavy metal exposure, thought to affect cardiovascular tissues through oxidative stress pathways, has similarly been linked to both atherosclerosis and DNA methylation [71]. In a study of children and young adults ($n \sim 700$), investigators observed that urinary concentrations of lead and cadmium were positively associated with both CIMT and global DNA methylation [72]. Similarly, in a pilot study of epigenetic changes in a cohort of middle-aged men ($n = 23$), 46% of DMRs associated with exposure to metals overlapped with atherosclerosis-related DMRs [73]. Pathway analyses showed that these genes were involved in inflammatory and metabolic processes. In a human cell line, treatment with arsenic exposure upregulated the transcription of DNA (cytosine-5)-methyltransferase 1 (*DNMT1*) via reactive oxygen species. The DNMT1 enzyme in turn methylated the *ABCA1* promoter, induced global hypomethylation, and inhibited cholesterol efflux in macrophages [74]. *ABCA1* is a member of the superfamily of ATP-binding cassette transporters that includes *ABCG1*, and its encoded protein plays a role in cholesterol efflux and HDL formation [75]. While investigators of these studies have proposed that DNA methylation may mediate the relationship between pollutants and lipids, further studies, such as Mendelian randomization, are needed to establish causality.

Diet

The relationship between methylation, diet, and lipids have been extensively explored. In the GOLDN study (n ~ 1000), CpG sites annotated to multiple genes, including *CPT1A*, were associated with postprandial lipemia—elevation of TG levels after eating a high-fat meal [33]. The association remained significant after adjusting for baseline TG levels for cg005794958 and cg1705847 (both annotated to *CPT1A*). Another EWAS in GOLDN found that increased methylation of the *ABCA1* promoter was associated with lower circulating omega-3 fatty acid and HDL levels [10]. Furthermore, a cross-sectional analysis of Japanese adults showed inverse relationships between *ABCA1* methylation and dietary vitamin intake. Investigators suggested that *ABCA1* methylation may mediate the effect of vitamin intake on HDL [76].

In a randomized controlled diet intervention trial, researchers evaluated the effect of a Mediterranean diet on methylation over a 5-year period [77]. Components of the Mediterranean diet induced methylation changes in genes associated with metabolism, diabetes, inflammation, and signal transduction. Specifically, Arpón et al. identified a CpG site (cg01081346) annotated to *CPT1B*—a paralog of *CPT1A* that encodes the rate-limiting enzyme of fatty acid oxidation in skeletal muscle—that was associated with polyunsaturated fatty acid intake among study participants. In another randomized, placebo-controlled trial of a dietary intervention, Lima et al. found that hazelnut oil consumption was associated with decreased methylation at *ADRB3* and an increase in HDL [78]. In a later EWAS, the research group found that *ADRB3* methylation was associated with higher fat intake and LDL [79]. *ADRB3* encodes the adrenoreceptor beta 3, which is involved in regulating lipolysis.

In both prospective and cross-sectional analyses across multiple populations, exposures to pollutants and endocrine disruptors (e.g., phthalates, heavy metals) have been linked to differential DNA methylation. Many of these genes are involved in lipid metabolism and homeostasis. Additionally, dietary factors, particularly the consumption of different types of fats, have also been associated with the methylation of these linked to lipid-related genes. Further investigation is needed to explain the complex relationship between environmental exposures, DNA methylation, and lipids.

Conclusion and Future Directions

In summary, studies suggest that DNA methylation, variation of plasma lipids, and pathogenesis of dyslipidemias are entwined. Associations have been validated across multiple cohorts (particularly for *CPT1A*, *ABCG1*, and *SREBF1*). Candidate gene and EWAS approaches are still broadly applied, but investigators are expanding their analyses to multi-omics approaches that include genomic, transcriptomic, and metabolomic data. Intervention trials that explore the effects of environmental factors, such as diet, have shown methylation variations in association with lipids. Still, large epigenomic studies are predominantly cross-sectional in design.

Future analyses should continue to increase the diversity of study populations, as European ancestry populations are disproportionately overrepresented in the current literature. Additionally, explanations of the causal effects of methylation on lipids (or vice versa) are lacking. More studies that apply approaches such as Mendelian randomization or that capture prospective data are needed to clarify the relationship between the environment, DNA methylation, and lipids. Studies should also continue to assess how these relationships alter gene expression across relevant tissues. Other considerations include the evaluation of methylation variation over the lifespan, or in the context of medication responses (i.e., pharmacoepigenetics). Overall, while there has been significant progress in our understanding of DNA

methylation and lipids, considerable research is still needed to translate this information into clinically applicable tools.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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•• Of major importance

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