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DNA methylation in human lipid metabolism and related diseases

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Purpose of review
It is becoming increasingly evident that epigenetic mechanisms, particularly DNA methylation, play a role in the regulation of blood lipid levels and lipid metabolism-linked phenotypes and diseases.

Recent findings
Recent genome-wide methylation and candidate gene studies of blood lipids have highlighted several robustly replicated methylation markers across different ethnicities. Furthermore, many of these lipid-related CpG sites associated with blood lipids are also linked to lipid-related phenotypes and diseases. Integrating epigenome-wide association studies (EWAS) data with other layers of molecular data such as genetics or the transcriptome, accompanied by relevant statistical methods (e.g. Mendelian randomization), provides evidence for causal relationships. Recent data suggest that epigenetic changes can be consequences rather than causes of dyslipidemia. There is sparse information on many lipid classes and disorders of lipid metabolism, and also on the interplay of DNA methylation with other epigenetic layers such as histone modifications and regulatory RNAs.

Summary
The current review provides a literature overview of epigenetic modifications in lipid metabolism and other lipid-related phenotypes and diseases focusing on EWAS of DNA methylation from January 2016 to September 2017. Recent studies strongly support the importance of epigenetic modifications, such as DNA methylation, in lipid metabolism and related diseases for relevant biological insights, reliable biomarkers, and even future therapeutics.

Keywords
DNA methylation, lipid metabolism, EWAS, blood lipids

INTRODUCTION: DNA METHYLATION AND BLOOD LIPID LEVELS - WHAT DO WE KNOW?

Abnormalities in the levels of circulating blood lipids, such as triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), contribute to the pathophysiology of common complex diseases, among them diabetes and cardiovascular diseases (CVDs) – two of the major causes of morbidity and mortality in industrialized countries [1–3]. Lipid disorders, also known as dyslipidemias, are primarily a result of unhealthy lifestyle choices: poor diet, lack of physical activity, and overweight, among others. Though these environmental factors are key contributors, the clustering of dyslipidemias in families has also been observed [4], which lends evidence for a genetic influence. Genome-wide association studies (GWAS) have identified a total of 157 common genetic loci associated with lipid levels, though combined these explain 12% or less of trait variance [5]. Consequently, evidence for epigenetic mechanisms playing a role in the regulation of lipid levels is being increasingly recognized. Unlike genetic variation, epigenetic modifications, such as DNA methylation, histone modification, and regulation by RNAs, are dynamically remodeled over time and can be affected

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KEY POINTS

- The current review provides an overview of literature summarizing the role of epigenetic modifications in lipid metabolism and lipid-related diseases with a focus on EWAS of DNA methylation from January 2016 to September 2017.
- Recent EWAS show that many CpG sites associated with blood lipids are also associated with lipid metabolism-linked phenotypes and diseases.
- The integration of DNA methylation data with other molecular layers accompanied by appropriate statistical methods improves our knowledge on the bidirectional interplay of lipids and methylation changes.

by environmental changes [6] and vary according to chromosomal location, alleles, type of cell, or phase of development [7,8]. This dynamism includes reversibility, making epigenetic modifications potentially important pathogenic mechanisms in complex metabolic diseases, and conceivably representing therapeutic targets [9▪].

Recent advances in omics technology allows a hypothesis-free search of epigenetic modifications, and, in particular, DNA methylation. These have helped identify new loci and pathways involved in lipid metabolism. Whereas there are more than five different DNA modifications known, the most widely studied is the transfer of a methyl group to the C5 position of a cytosine to form a 5-methylcytosine. In conjunction with human lipid traits, DNA methylation is by far the most studied epigenetic process [9▪,10]. Epigenome-wide association studies (EWAS) have become a powerful instrument to investigate differences in DNA methylation at the population level. Regarding lipid levels, EWAS have highlighted several robustly replicated methylation markers such as cg06500161, annotated to the *ABCG1* gene encoding ATP-binding cassette subfamily G member 1 and cg00574958 within *CPT1A* gene encoding carnitine palmitoyltransferase I.

Petersen *et al.* [11] conducted an EWAS of metabolic traits in whole blood and identified associations between multiple lipids (including cholesterol, sphingolipids, and glycerophospholipids) and lipoproteins, and the methylation level of CpG sites in or in close proximity to the genes 24-dehydrocholesterol reductase (*DHCR24*), thioredoxin-interacting protein (*TXNIP*), solute carrier family 22 member 25 (*SLC25A22*), *CPT1A,* myosin VC (*MYO5C*), and *ABCG1* [11]. Irvin *et al.* [12] reported that four CpG sites in intron 1 of *CPT1A* were strongly associated with very-low to low-density lipoprotein cholesterol (VLDL-C) and triglycerides. They also showed an inverse association between *CPT1A* methylation (cg00574958) and expression of *CPT1A*. A further EWAS – Frazier-Wood *et al.* [13] – in CD4+ T cells revealed associations between LDL-C and VLDL-C levels, and methylation of CpG sites in *CPT1A* [13]. The results were later replicated in blood by Gagnon *et al.* [14]. Pfeiffer *et al.* [15] reported associations in whole blood between DNA methylation and triglycerides for CpG sites mapping to the genes *CPT1A, ABCG1, SREBF1* encoding sterol regulatory element-binding transcription factor 1 and the *SCD* gene encoding stearoyl-CoA desaturase, between DNA methylation and HDL-C for a CpG in *ABCG1*, and between DNA methylation and LDL-C for a CpG in *TXNIP1*. Most of the above reported genes have an important function in lipid metabolism, supporting the hypothesis that epigenetic changes play regulatory roles. Furthermore, several EWAS of lipid-related metabolic phenotypes and diseases, for example, those for BMI, waist circumference [16–19], and type 2 diabetes (T2D) [20–22], have uncovered associations with many of the same CpG sites. In this review, we will summarize the latest results from January 2016 to September 2017 concerning EWAS of DNA methylation and lipid traits, and also lipid-related disease.

NEWLY DISCOVERED CPG SITES AND THEIR LEVEL OF EVIDENCE

Recent EWAS and candidate gene studies have been able to confirm the strong associations reported above between various CpG sites and blood lipid levels across different ethnicities (Tables 1 and 2) [23▪▪,24▪,25–27,28▪▪,29–31]. Furthermore, they have shown that many CpG sites associated with blood lipids are also associated with lipid metabolism-linked phenotypes and diseases (Table 2). Recently, Hedman *et al.* [24▪] reported 25 novel CpG sites not previously found to be associated with lipid levels. The annotated genes were enriched in pathways involved in lipid and amino acid metabolism [24▪]. Methylation levels at *ABCG1* (cg27243685) were additionally reported in relation to occurrence of CVD events [24▪]. The authors further showed that triglyceride levels were associated with DNA methylation in the serine metabolism gene *PHGDH* encoding D-3-phosphoglycerate dehydrogenase (cg14476101), a result confirmed by Truong *et al.* [30]. Public database findings support a functional role of cg1476101 in *PHGDH* expression [30].

Wahl *et al.* [28▪▪] identified methylation loci associated with BMI in genes [e.g. *CPT1A, DHCR24, SREBF1*, and *SOCS3* (suppressor of cytokine signaling

Table 1. Epigenome-wide association studies (EWAS) of DNA methylation and lipid traits

Annotated genes	CpG sites	Chr	TG	HDL-C	LDL-C	TC	Reference	Previously associated with
CPT1A[a]	cg00574958 cg17058475 cg09737197 cg01082498	11	●				Dekkers *et al.* [23▪▪] Braun *et al.* [25] Sayols-Baixeras *et al.* [38▪▪] Hedman *et al.* [24▪]	**TG, LDL-C** (Pfeiffer *et al.* [15] Irvin *et al.* [12])
IGFBP5	cg00011856	2	●				Tremblay *et al.* [26]	
ATF1	cg05655647	12	●				Tremblay *et al.* [26]	
SARS[a]	**cg03725309**	1	●				Hedman *et al.* [24▪]	
PHGDH	cg16246545	1	●				Hedman *et al.* [24▪] Truong *et al.* [30]	**BMI** (Aslibekyan *et al.* [19])
TXNIP	cg19693031	1	●				Hedman *et al.* [24▪] Sayols-Baixeras *et al.* [38▪▪] Dayeh *et al.* [31]	**TG** (Pfeiffer *et al.* [15])
SLC7A11	cg06690548	4	●				Hedman *et al.* [24▪] Sayols-Baixeras *et al.* [38▪▪]	
GARS	cg03068497	7	●				Hedman *et al.*[24▪]	
VPS25	cg08857797	17	●				Hedman *et al.*[24▪]	**BMI** (Demerath *et al.* [16])
SLC1A5[a]	cg2711608	19	●				Hedman *et al.*[24▪]	
MYLIP[a]	cg03717755	6	●				Sayols-Baixeras *et al.* [38▪▪]	**T2D** (Kulkarni *et al.* [22])
SREBF1[a]	cg11024682 cg08129017	17	●	●			Dekkers *et al.*[23▪▪] Braun *et al.* [25] Hedman *et al.* [24▪] Sayols-Baixeras *et al.* [38▪▪]	**TG** (Pfeiffer *et al.* [15])
ABCG1[a]	cg06500161 cg27243685 cg01881899 cg02370100 cg01176028	21	●	●			Hedman *et al.* [24▪] Braun *et al.* [25] Dekkers *et al.* [23▪▪] Sayols-Baixeras *et al.* [38▪▪] Truong *et al.* [30] Dayeh *et al.* [31]	**TG, HDL-C** (Pfeiffer *et al.* [15]) **BMI** (Arner *et al.* [18])
SOCS3[a]	cg18181703	17	●	●			Ali *et al.* [27]	
DHCR24[a]	cg17901584 cg27168858	1		●	●	●	Braun *et al.* [25] Dekkers *et al.* [23▪▪] Hedman *et al.* [24▪]	
SREBF2[a]	cg09978077 cg16000331	22				●	Hedman *et al.* [24▪] Sayols-Baixeras *et al.* [38▪▪]	
OXER1	cg23759710	2				●	Hedman *et al.* [24▪]	
SQLE	cg00285394	8			●	●	Hedman *et al.* [24▪]	
NLRC5	cg07839457	16				●	Hedman *et al.* [24▪]	
GATAD2B	cg07567724	1		●			Hedman *et al.* [24▪]	
PIKFYVE	cg19351166	2		●			Hedman *et al.* [24▪]	
NFKBIE	cg06560379	6		●			Hedman *et al.* [24▪]	
UFM1	cg19750657	13		●			Hedman *et al.* [24▪]	
KLF13	cg07814318	15		●			Hedman *et al.* [24▪]	**BMI** (Demerath *et al.* [16])
MYO5C	cg06192883	15		●			Hedman *et al.* [24▪]	**BMI, WC** (Demerath *et al.* [16])
SPRY4	cg06397161	5		●			Hedman *et al.* [24▪]	
PHOSPHO1	cg02650017	17		●			Sayols-Baixeras *et al.* [38▪▪] Dayeh *et al.* [31]	
SYNGAP1	cg09572125	6		●			Sayols-Baixeras *et al.* [38▪▪]	

CpGs and annotated genes in bold are also described in the literature as associated with lipid phenotypes and/or lipid-related diseases (Table 2). All associations were investigated in blood.
Chr, chromosome; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2D, type 2 diabetes; TC, total cholesterol; TG, triglyceride; WC, waist circumference.
[a]Expression data are available.

3)] that are involved in lipid metabolism [28▪▪]. These associations between BMI and lipid-related CpG sites were confirmed by additional studies in Arab and European populations [32,33▪▪,34]. It was additionally uncovered that the SOCS3 methylation locus is associated with multiple metabolic syndrome traits, including central obesity, fat depots, insulin responsiveness, and plasma lipids (HDL-C and triglycerides) [27,35]. Furthermore, *SOCS3* was found to be associated with lipid levels and insulin resistance in human GWAS and candidate gene studies [36]. Recent EWAS, conducted in Indian, Arab, and

Table 2. Epigenome-wide association studies of DNA methylation and lipid phenotypes or lipid related diseases

Annotated genes	CpG sites	Chr	BMI	BMI%	MetS	HTGW	TG-PPL	T2D	Reference	Previously associated with
CPT1A[a]	**cg00574958 cg17058475**	11	●		●	●	●		Mendelson *et al.* [33▪▪] Al Muftah *et al.* [32] Das *et al.* [42] Mamtani *et al.* [41] Lai *et al.* [40] Wahl *et al.* [28▪▪]	**TG, LDL-C** (Pfeiffer *et al.* [15] Irvin *et al.* [12]) **BMI** (Demerath *et al.* [16] Aslibekyan *et al.* [19]) **T2D** (Kulkarni *et al.* [22])
ABCG1[a]	**cg06500161 cg27243685 cg01881899 cg10192877**	21	●			●	●		Mendelson *et al.* [33▪▪] Wilson *et al.* 2017 Mamatani *et al.* [41] Lai *et al.* [40] Wahl *et al.* [28▪▪] Dayeh *et al.* [31]	**TG, HDL-C** (Pfeiffer *et al.* [15]) **BMI, WC** (Demerath *et al.* [16]) **T2D** (Chambers *et al.* [20] Kulkarni *et al.* [22])
DHCR24[a]	**cg17901584**	1	●						Mendelson *et al.* [33▪▪] Wahl *et al.* [28▪▪] Wilson *et al.* [34]	**WC** (Demerath *et al.* [16])
SARS	**cg03725309**	1	●						Mendelson *et al.* [33▪▪]	
SLC1A5	**cg02711608**	19	●						Mendelson *et al.* [33▪▪]	
SREBF1[a]	**cg11024682**	17	●				●		Mendelson *et al.* [33▪▪] Al Muftah *et al.* [32] Lai *et al.* [40] Wahl *et al.* [28▪▪] Dayeh *et al.* [31]	**TG** (Pfeiffer *et al.* [15]) **BMI, WC** (Demerath *et al.* [16]) **T2D** (Chambers *et al.* [20] Kulkarni *et al.* [22])
SOCS3[a]	**cg18181703**	17	●	●	●			●	Ali *et al.* [27] Al Muftah *et al.* [32] Wahl *et al.* [28▪▪] Dayeh *et al.* [31] Wilson *et al.* [34]	**T2D** (Chambers *et al.* [20])
TXNIP[a]	**cg19693031**	1						●	Florath *et al.* [37] Al Muftah *et al.* [32]	**TG** (Pfeiffer *et al.* [15]) **T2D** (Chambers *et al.* [20] Kulkarni *et al.* [22])
MYO5C	**cg06192883**	15	●						Wahl *et al.* [28▪▪]	**BMI, WC** (Demerath *et al.* [16])
SBNO2	cg07573872	19	●						Al Muftah *et al.* [32] Wahl *et al.* [28▪▪]	**BMI** (Demerath *et al.* [16])
PRR5L	cg07136133 cg00220721	11	●						Al Muftah *et al.* [32] Wahl *et al.* [28▪▪]	**BMI** (Demerath *et al.* [16])
APOA5	**cg12556569**	11					●		Lai *et al.* [40]	**TG** (Pfeiffer *et al.* [15])
LPP	cg16464007	3	●				●		Wahl *et al.* [28▪▪] Lai *et al.* [40]	
LY6G6E	cg13123009	6	●						Al Muftah *et al.* [32]	**BMI, WC** (Demerath *et al.* [16])
SMARCA4	cg22898082 cg17218495	19	●						Wahl *et al.* [28▪▪]	
KLF13	**cg07814318**	15	●						Wahl *et al.* [28▪▪]	**BMI** (Demerath *et al.* [16])
UFM1	**cg19750657**	13	●						Wahl *et al.* [28▪▪]	
VPS25	**cg08857797**	17	●						Wahl *et al.* [28▪▪]	**BMI** (Demerath *et al.* [16])
HOXA3	cg01964852	7	●						Wahl *et al.* [28▪▪]	
SYNGAP1[a]	cg22740603	6	●						Wahl *et al.* [28▪▪]	
PHOSPHO1[a]	**cg02650017**	17	●						Wahl *et al.* [28▪▪]	
SPRY4	**cg13305415**	5	●						Wahl *et al.* [28▪▪]	
NFKBIE	**cg06560379**	6	●						Wahl *et al.* [28▪▪]	
PIKFYVE	**cg19351166**	2	●						Wahl *et al.* [28▪▪]	
SLC7A11[a]	cg07661704	4	●						Wahl *et al.* [28▪▪]	
PHGDH	cg14476101	1	●						Wahl *et al.* [28▪▪]	
IGFBP5	cg05485437	2	●						Wahl *et al.* [28▪▪]	
MYLIP[a]	**cg03717755**	6	●						Wahl *et al.* [28▪▪]	**T2D** (Kulkarni *et al.* [22])
CACNA2D3[a]	cg01368219	3	●						Mendelson *et al.* [33▪▪]	
RPS6KA2	cg17501210	6	●						Wilson *et al.* [34] Wahl *et al.* [28▪▪]	

Table 2 (*Continued*)

Annotated genes	CpG sites	Chr	BMI	BMI%	MetS	HTGW	TG-PPL	T2D	Reference	Previously associated with
FSD2	cg07728579	15	●						Wilson *et al.* [34] Wahl *et al.* [28▪▪]	
STK39	cg11775828	2	●						Wilson *et al.* [34]	
CRHR2	cg13134297	7	●						Wilson *et al.* [34]	
ZNF771	cg04502490	16		●					Ali *et al.* [27]	
LIMD2	cg02988947	17		●					Ali *et al.* [27]	

CpGs and annotated genes in bold are also described in the literature as associated with blood lipids (Table 1).
Chr, chromosome; HDL-C, high-density lipoprotein cholesterol; HTGW, hypertriglyceridemic waist; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; T2D, type 2 diabetes; TC, total cholesterol; TG-PPL, triglyceride postprandial responses; TG, triglyceride; WC, waist circumference.
[a]Expression data are available.

Caucasian populations, found that *SOCS3* methylation is associated with BMI and T2D, respectively [20,32,34]. Another interesting methylation site *(TXNIP,* cg19693031) associated with T2D in several studies [20,22,32,37] was also reported to be associated with triglyceride and LDL-C levels [15,24▪,38▪▪].

Differential DNA methylation of five CpG sites annotated to *ABCG1, PHOSPHO1* (phosphoethanolamine/phosphocholine phosphatase), *SOCS3*, *SREBF1*, and *TXNIP* from diabetic versus nondiabetic patients were investigated across different tissues from the same individuals [31]. The results suggest that DNA methylation biomarkers in blood might partly be used as surrogate markers for DNA methylation in inaccessible target tissues, and, importantly, the occurrence of altered DNA methylation in more than one human tissue at the same locus could be mediated by so-called 'metastable epialleles' [31]. Metastable epialleles are alleles that are variably expressed in genetically identical individuals due to epigenetic modifications that were established during early development [39]. BMI-related methylation markers identified by Wahl *et al.* [28▪▪] were strongly enriched for CpG sites with intermediate levels of methylation, consistent with the presence of mosaicism, that is, epigenetic heterogeneity, at these loci. The authors performed replication testing in isolated white cell subsets (monocytes, neutrophils, CD4+ T cells, and CD8+ T cells), showing that epigenetic heterogeneity was present at the majority of loci, in each of the cell subsets studied [28▪▪]. Wahl *et al.* [28▪▪] compared methylation levels between blood, subcutaneous and omental fat, liver, muscle, spleen, and pancreas. Mean methylation levels at the 187 loci correlated moderately to strongly between the tissues, supporting the view that methylation levels in blood are related to methylation patterns in other tissues at the CpG sites examined.

Lai *et al.* [40] showed that eight methylation sites encompassing different genes *LPP* encoding lipoma-preferred partner, *APOA5* encoding apolipoprotein A-V, *SREBF1*, *ABCG1*, and *CPT1A* were associated with triglyceride postprandial responses (TG-PPL), an independent CVD risk factor, after consuming a high-fat meal [40]. These genes had been previously found to be associated with triglyceride and/or HDL-C levels [15,23▪▪,24▪,25,38▪▪]. Data from a Mexican-American study showed cg00574958 and cg17058475 (*CPT1A)* and cg06500161 (*ABCG1*) to be associated with hypertriglyceridemic waist (HTGW), which is defined as large waist circumference combined with high serum triglyceride concentration [41]. Both CpG sites in *CPT1A* were additionally associated with the metabolic syndrome in CD4+ T cells [42]. Recently, *CPT1A* methylation status was also found to be significantly associated with plasma adiponectin, a widely used biomarker for cardiovascular and metabolic risk [43▪].

So far, EWAS on disorders of lipid metabolism are sparse [44,45]. Sitosterolemia is a rare autosomal recessive sterol storage disease caused by mutations in either of the adenosine triphosphate binding cassette transporter genes *ABCG5* or *ABCG8* encoding ATP-binding cassette subfamily G member 5 or 8, leading to substantially elevated serum plant sterols with moderate to high total cholesterol and LDL-C levels and increased risk of premature atherosclerosis [46]. Interestingly, *ABCG5* methylation was associated with lower LDL-C and reduced risk for coronary artery disease (CAD) [47,48]. In the study by Rask-Andersen *et al.* [47], a total of 6 out of 211 myocardial infarction-associated CpG sites overlapped with previously identified CVD GWAS loci, among them the *ABCG5-ABCG8* locus [47]. The investigation into further lipid classes and studies on disorders of lipid metabolism will provide new and important insights.

CROSS-OMICS: EVIDENCE FROM ADDITIONAL MOLECULAR LAYERS

Different molecular layers often have complementary roles to jointly perform a certain biological function [49]. Population-based studies adopted the multiomics approach by integrating these molecular layers into their studies. Whereas this approach has been successfully used for available transcriptome, metabolome, or genetic data, studies are sparse that systematically investigate the interaction of epigenetic mechanisms such as regulatory RNAs or histone modifications [50].

LIPID-ASSOCIATED METHYLATION QUANTITATIVE TRAIT LOCI AND REGULATION OF GENE EXPRESSION

The variance of lipid levels explained by the currently known genetic variants is modest. All lipid-associated single-nucleotide polymorphisms (SNPs) together explain 12% or less of the variation in plasma lipid traits [5], although the estimated heritable variance of lipids is reported to be at least 50% [51]. This missing heritability may be partly explained by epigenetic processes such as DNA methylation [52]. SNP allele frequencies are known to differ among populations with varying geographic ancestries, suggesting that ethnic differences in DNA methylation could be due to differences in population-specific alleles that shape CpG and global methylation levels. Regulation of gene expression via DNA methylation may explain an additional component of interindividual variation in lipid levels beyond genetic sequence variants. Linking DNA methylation data with gene expression is a promising avenue to see potential downstream effects in lipid metabolism.

Hedman *et al.* [24▪] found methylation levels of lipid-related CpG sites associated with mRNA expression levels of nearby genes, including cg17901584 (*DHCR24*), cg14476101, cg16246545 (both *PHGDH*), and cg08129017 (*SREBF1*). For the majority (86%) of these associations, levels of methylation and expression were inversely correlated [24▪]. In agreement with previous studies, they found a large proportion of lipid-related CpG sites to associate with common SNPs in cis. For 12 CpG-transcript pairs, a cis-meQTL was identified and the lead meQTL SNP was significantly associated with both methylation and expression [24▪].

Volkov *et al.* [35] described methylation quantitative trait loci (meQTLs) in adipose tissue. These meQTLs include reported obesity, lipid, and T2D loci, for example, APOA5, cholesteryl ester transfer protein (CETP), and fatty acid desaturase 2 (FADS2). SNPs in significant meQTLs were also associated with BMI, lipid traits, and glucose and insulin levels [35]. The meQTL at the *APOA5* loci was confirmed by Oliva *et al.* [53] using a candidate gene approach.

Ali *et al.* [27] assessed the relationship between DNA methylation, obesity, and obesity-related phenotypes in peripheral blood mononuclear cells. They found that the methylation status of cg18181703 (*SOCS3*) significantly alters *SOCS3* gene expression [27,35]. Using RNA-seq data, DNA methylation of six CpG sites was associated with the expression of *CPT1A* and *SREBF1* (for triglycerides), *DHCR24* (for LDL-C), and *ABCG1* (for HDL-C) [23▪▪]. The results could be confirmed by Braun *et al.* [25]. For *CPT1A*, expression was negatively associated with the methylation of *CPT1A* at both identified CpG sites (cg00574958 and cg17058475). A study by Bekkering *et al.* [54] showed that the expression of lipid metabolism genes were altered after oxidized LDL exposure of monocytes. Methylation of CpG sites within exon 3 of APOA5 was positively correlated with triglyceride concentration and with a lipoprotein profile associated with atherogenic dyslipidemia [53]. Another candidate gene study reported decreased methylation levels of the actin-related protein 2/3 complex subunit 3 (*ARPC3*) promoter-associated CpG site cg10738648 in both visceral adipose tissue and blood for carriers of the rs3759384 T allele in obese patients with hypertriglyceridemia, and showed *ARPC3* expression to be correlated with plasma triglyceride levels [55]. Finally, lower *TNNT1* DNA methylation levels were found to be independently associated with lower HDL-C levels and a *TNNT1* polymorphism in patients with and without familial hypercholesterolemia [29]. Genetic variations of the *TNNT1* locus have previously been associated with HDL-C levels in several GWAS [36].

MENDELIAN RANDOMIZATION: A TOOL FOR CAUSAL INFERENCE IN DNA METHYLATION STUDIES

To determine whether lipids influence DNA methylation or DNA methylation causes differences in lipid levels, Mendelian randomization was put forward as a tool for causal inference in DNA methylation studies [56,57]. Although Mendelian randomization can provide strong evidence for causal relationships, the quality of evidence provided by a Mendelian randomization study heavily relies on the underlying assumptions [58]. Applications and limitations of Mendelian randomization in EWAS have been recently reviewed [59].

Dekkers *et al.* [23▪▪] showed that differential methylation is the consequence of interindividual variation in blood lipid levels and not *vice versa*.

Using multivariate Mendelian randomization, they reported an effect of blood lipids on DNA methylation at six CpG sites. A large-scale EWAS in peripheral blood reported by Mendelson *et al.* [33▪▪] identified associations between BMI and methylation at 83 replicated CpG sites, with an over-representation of lipid metabolism pathways among those CpG sites associated with gene expression changes. Eleven CpG sites revealed three-way associations, whereby DNA methylation was associated with BMI and expression, and also with BMI-associated expression changes, including the known lipid-related CpG sites within *ABCG1*, *CPT1A*, *DHCR24*, *SLC1A5*, and *SREBF1*. Using Mendelian randomization, 16 CpG sites were found to be differentially methylated as a consequence of BMI [33▪▪]. These 16 CpG sites were annotated to 12 genes, including *ABCG1*. Among the 83 BMI-related CpG sites, only cg11024682 (*SREBF1*) showed evidence for a causal effect on BMI. Genetically predicted exposure to differential methylation and *SREBF1* gene expression was associated with dyslipidemia, adiposity-related traits, and CAD [33▪▪]. Wahl *et al.* [28▪▪] subsequently showed in whole blood and adipose tissue that DNA methylation at lipid-related CpG sites is predominantly the consequence of adiposity and not the cause. Whereas Dekkers *et al.* [23▪▪] suggest that methylation of cg11024682 (*SREBF1*) is induced by triglyceride levels, the analysis of Mendelson *et al.*'s [33▪▪] study reports a causal effect of the same CpG site on BMI, a result not confirmed by Wahl *et al.* [23▪▪,28▪▪,33▪▪]. All recently conducted Mendelian randomization studies, however, highlight the causal effect of methylation at the *ABCG1* loci on both BMI and lipid levels [23▪▪, 28▪▪,33▪▪].

CONCLUSION AND FUTURE DIRECTIONS

Epigenetics continues to be a promising area of research in lipid-related diseases. Current scientific knowledge does not completely explain the molecular mechanisms behind lipid metabolism and lipid-related diseases. Epigenetic modifications, such as DNA methylation, might form an additional path to understanding the mechanisms of lipid-related diseases. However, many challenges regarding the design, conduct, and interpretation of EWAS persist. The main challenges include accounting for variation in cellular heterogeneity, potential confounding effects, and resolving whether blood samples do indeed mirror relevant targeted tissues. Therefore, longitudinal cohort studies and larger sample sizes are key points for further investigations. Moreover, in addition to the development of cost-effective sequencing applications, a new array has been developed covering more than 850 000 methylation sites across the genome.

Investigation into further lipid classes, beyond the traditional blood lipids, and studies on disorders of lipid metabolism will provide new and important insights. Furthermore, other epigenetic layers need to gain importance, for example, the interplay between microRNAs and other epigenetic regulators such as histone modifications and DNA methylation. For example, it is becoming increasingly evident that post-transcriptional repression by microRNAs, a class of small noncoding RNAs, is a key layer of regulation in several biological processes, including lipid phenotypes [60]. The NIH Roadmap Epigenomics Consortium has generated a large collection of human epigenomes for primary cells and tissues, describing the integrative analysis of 111 reference human epigenomes generated as part of the program, profiled for histone modification patterns, DNA accessibility, DNA methylation, and RNA expression, providing a unique resource for such investigations [61].

Another important task is to assess, and functionally validate, causality of the reported associations, and, if we propose that a change in DNA methylation status is causal for a lipid phenotype, to assess when these changes occur [62]. For example, it has been indicated that for a growing fetus, malnutrition can have harmful effects on prenatal programming and contribute to the development of diseases later in life [63,64]. Perhaps, the greatest challenge is to understand the functional consequences of the confirmed loci. Biological insights can then be translated to clinical benefits, including reliable biomarkers and effective strategies for disease prevention. Functional follow-up studies of confirmed loci will help unravel the precise molecular mechanisms at specific CpG sites, including the identification of methylation-specific binding proteins and characterization of their mode of action.

Although knowledge of epigenetic changes, such as DNA methylation, has the potential to shed light on the differences in lipid concentrations and the underlying pathways' mechanisms, the ultimate goal remains the translation of this knowledge into the effective prediction and treatment of lipid-related diseases.

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Conflicts of interest

There are no conflicts of interest.

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