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Epigenetics of Lipid Phenotypes

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Abstract

Dyslipidemia is a well-established risk factor for cardiovascular disease, the main cause of death worldwide. Blood lipid profiles are patterned by both genetic and environmental factors. In recent years, epigenetics has emerged as a paradigm that unifies these influences. In this review, we have summarized the latest evidence implicating epigenetic mechanisms—DNA methylation, histone modification, and regulation by RNAs—in lipid homeostasis. Key findings have emerged in a number of novel epigenetic loci located in biologically plausible genes (e.g. *CPT1A, ABCG1, SREBF1*, and others), as well as microRNA-33a/b. Evidence from animal and cell culture models suggests a complex interplay between different classes of epigenetic processes in the lipid-related genomic regions. While epigenetic findings hold the potential to explain the interindividual variability in lipid profiles as well as the underlying mechanisms, they have yet to be translated into effective therapies for dyslipidemia.

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors

Conflicts of interest

Drs Vaillant, Kolden & Smith declare that they have no conflicts of interests

Keywords

Epigenetics; Methylation; Lipids; Triglycerides; Cholesterol

Introduction

Elevated plasma triglycerides (TGs), alone or in conjunction with increased low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) cholesterol, are well-documented risk factors for cardiovascular disease [1]. Despite high estimates of heritability (48% and above [2]) and limited influence of shared environment, known genetic variants explain approximately 10% of the observed variance in plasma lipids [3, 4]. Recent evidence shows that a significant proportion of the remaining genetic variability may be attributable to epigenetic processes, defined as heritable, non-sequence-dependent changes in gene expression [5].

In contrast to sequence polymorphisms, epigenetic variation is specific, dynamic (and even reversible throughout the lifecourse), and mechanistically diverse. These characteristics both make it a lucrative target and pose translational challenges. For example, the cell and tissue specificity enhances pathophysiologic relevance, but often complicates measurements as probing some organs implicated in lipid metabolism—e.g. the liver—is invasive and costly. While several studies have demonstrated conservation of epigenetic patterns and validated the use of easily accessible tissues such as blood for epigenetic measurements [6], the variants that remain tissue-specific are more likely to be causally related to disease risk. Furthermore, the reversibility of epigenetic marks raises the issue of reverse causation: is the change in the epigenetic profile a cause or a consequence of the phenotype? Despite plausible mechanisms for either possibility in the lipid context, current evidence for DNA methylation supports only the latter path, wherein lipids prime circulating immune cells to regulate their own synthesis [7]. Finally, the sheer variety of biochemical modifications involved in epigenetic inheritance creates a daunting yet promising ground for investigations. Known processes that contribute to epigenetic effects are chromatin remodeling via DNA methylation and histone modification, RNA-based mechanisms, structural inheritance (or three-dimensional templating), and self-sustaining metabolic loops (reviewed in [8]). Currently available evidence supports the diverse roles of DNA methylation, histone modification, and regulatory RNAs in the setting of dyslipidemia; these three classes of epigenetic mechanisms will constitute the main focus of this review.

DNA methylation

DNA methylation, which refers to the addition of a methyl group to the DNA molecule, is by far the most studied epigenetic process with respect to human lipid traits, with several large-scale epigenome-wide studies enabled by cost-effective methylation array technology. In human somatic cells, DNA methylation occurs at approximately 70%–80% of cytosine-phosphase-guanine (CpG) sites throughout the genome [9] and can be influenced by both DNA sequence and environmental inputs. Depending on the location of the CpG site and the physiologic context, methylation can down- or upregulate gene expression via a variety of

mechanisms, including but not limited to effects on chromatin conformation, alternative splicing, and mRNA transcript levels (reviewed in [10]).

The emerging body of evidence linking lipids and DNA methylation includes three categories: 1) cross-sectional studies fasting lipids; 2) epigenetic predictors or correlates of lipid changes following a dietary or a pharmaceutical intervention; and 3) transgenerational effects of maternal genotype and/or environment on offspring lipid levels. Fasting lipids are a standard measurement in many epidemiologic cohorts and most early epigenetic studies have focused on candidate gene or repetitive element methylation (Table 1). The most robust and interesting targets, however, have arisen from epigenome-wide investigations of fasting lipids (Table 2). The earliest such study reported two differentially methylated sites in *TNNT1* using the Illumina 27K technology in participants with familial hypercholesterolemia [11]. However, these findings were not replicated in subsequent studies using the higher-resolution Illumina 450K chip. Instead, the next wave of studies pointed to methylation in the first intron of *CPT1A*, which encodes the liver isoform of carnitine palmitoyltransferase 1 (CPT1), a key enzyme in the fatty acid biosynthesis pathway [12]. A deficiency in CPT1 impairs the carnitine-dependent transport of long-chain fatty acids into the mitochondria, lowering the rate of beta-oxidation [13] with profoundly adverse metabolic effects [14]. In the first published epigenome-wide study of fasting lipids using the 450K array, Irvin *et al.* have reported a robust inverse association between *CPT1A* methylation and expression as well as fasting TGs and very low density lipoprotein (VLDL) cholesterol [12]. Methylation at the top *CPT1A* locus explained 12% of TG variation in the discovery cohort (Genetics of Lipid Lowering Drugs and Diet Network (GOLDN), CD4+ lymphocytes, n=991) and 6% in the replication cohort (Framingham Heart Study, whole blood, n=1261) [12]. Subsequent studies have replicated the observed association with lipids [7, 15, 16] and lipoprotein subfraction profiles [17], as well as linked *CPT1A* methylation with obesity traits [18, 19], metabolic syndrome [20], and hypertriglyceridemic waist [21] in diverse populations. Interestingly, *CPT1A* loci were also shown to be differentially methylated in Dutch Hunger Winter survivors exposed to famine *in utero*, associating with both birth weight and serum LDL cholesterol levels later in life [22] and providing a possible mechanism for the well-known adverse metabolic sequelae of prenatal malnutrition.

Another robust association with multiple phenotypes, namely increased TGs and markers of insulin resistance and decreased HDL-cholesterol, was reported in multiple cohorts and tissues for a methylation locus in *ABCG1* [15, 23]. Similarly to the *CPT1A* finding, the observed association exhibits strong biological plausibility because the product of *ABCG1* plays a central role in cholesterol and phospholipid reflux [24]. The differential methylation pattern of *ABCG1* with respect to HDL cholesterol and TGs, potentially mediated by *ABCG1* expression, was observed in both blood and adipose tissue but not skin, and putatively associated with prevalent myocardial infarction [15]. The same epigenome-wide study also identified and replicated associations between TGs and methylation loci in *MIR33B/SREBF1* and an intergenic region on chromosome 10, and between LDL cholesterol and a locus in *TNIP1* [15]. *SREBF1*, which contains intronic microRNA (miR)33b, and *SREBF2* encode transcription factors that regulate fatty acid and cholesterol metabolism [25]. Notably, miR33b represses the transcription of both *CPT1A* and *ABCG1* (reviewed in [15]), demonstrating the interplay between 1) genes that exhibit differential

methylation in dyslipidemia and 2) various classes of epigenetic processes, in particular methylation and RNA-based mechanisms. The first type of interplay is also illustrated by the connection between the LDL-cholesterol locus in *TNIP1*, the gene encoding a protein that interacts with tumor necrosis factor alpha, and *ABCG1* via the peroxisome proliferator-activated receptors (reviewed in [15]).

Most reports of associations between methylation variants and lipid phenotypes in humans came from cross-sectional studies, which preclude any inferences of temporality and thus causality. The few dynamic studies of DNA methylation have yielded mixed results. In the GOLDN cohort, there was no evidence that the changes in lipids that occur over the 3-week long treatment with fenofibrate correlate with either epigenome-wide or candidate gene methylation changes [26]. In the same study population, however, TG response to a high-fat meal intervention was linked to differential methylation of *CPT1A, APOA5, LPP, SREBF1*, and *ABCG1* [unpublished], which collectively explained approximately 15% of the phenotypic variance; however, the association was attenuated after adjustment for fasting levels. Nevertheless, these epigenetic markers—three of which overlap with known methylation correlates of fasting lipids— merit follow-up studies on the basis of their functional plausibility. Other prospective evidence comes from transgenerational studies that highlight the importance of the intrauterine environment for the risk of impaired metabolism in the offspring [27, 28]. These studies, however, are few and limited by the lack of mechanistic insights [28], repeated methylation measurements throughout the life course [22], or both.

The best evidence of causal relationships between lipids and DNA methylation comes from a recent epigenomic study of lipids in whole blood from 3296 individuals that employed stepwise Mendelian randomization, an instrumental variable approach which uses genotype at a validated locus (e.g. a lipids genetic risk score) as a randomly assigned proxy for the phenotype itself [7]. The advantages of Mendelian randomization, and particularly Egger regression (MR-Egger) as implemented by Dekkers *et al.*, include control for measured and unmeasured confounding and pleiotropy [29]. Using the MR-Egger approach, this largest epigenome-wide investigation of lipids to date has generated compelling evidence that lipids influence differential methylation in *CPT1A, SREBF1* (TGs), *ABCG1* (TGs, HDL cholesterol), and *DHCR24* (LDL cholesterol), but not vice versa [7]. Of the four genes causally implicated by MR-Egger, only *DHCR24* had not been previously discussed in cross-sectional studies. The *DHCR24* gene is nevertheless highly relevant because it encodes the enzyme catalyzing the terminal step in the cholesterol biosynthesis pathway [30], and is regulated by sterol regulatory element binding proteins such as those encoded by *SREBF1* [31]. The conclusions of the study, which support the causal effects of lipids on methylation at several biologically plausible loci, are consistent with prior evidence of cholesterol inhibiting its own synthesis—possibly via epigenetic priming that occurs prior to tissue differentiation [32, 33, cited in 7]. Future studies would benefit from interrogating other germane tissues, e.g. samples from adipose or hepatic biopsies, to identify a comprehensive methylation signature for dyslipidemia. Finally, it is worth remarking that of all the genes reported to present differential methylation associated with lipid traits, only some have been identified in genome-wide association studies to be associated with these traits (e.g. *CPT1A,*

MYLIP, and *APOA5*), highlighting the potential of epigenome-wide association studies to explain the "missing heritability" of lipid metabolism [34, 35].

Histone modifications

Post-translational modifications of diverse amino acid residues on histones, which include acetylation, methylation, and phosphorylation, represent another class of epigenetic mechanisms governing gene expression [36]. While chromatin immunoprecipitation (ChIP) methods can be used to characterize histone modifications in selected regions (via quantitative PCR) or on the epigenome-wide scale (via arrays or deep sequencing), most existing studies of lipid phenotypes use this technology for follow-up of DNA sequence or methylation findings rather than discovery. For example, functional annotation of 95 known lipid SNPs [34] with data from the Encyclopedia of DNA Elements (ENCODE) project found enrichment for histone marks associated with transcription regulation, particularly in hepatic cells [37]. A similar analysis of SNPs in *GALNT2*, an HDL cholesterol locus, found multiple instances of overlap with histone modification peaks in the liver [38]. This approach may be especially promising in DNA methylation studies due to the cross-talk between enzymes involved in both histone and DNA modifications [39]. The interdependency of epigenetic processes is observed in all four genes harboring lipid-regulated methylation loci, as the expression of *DHCR24* [40], *CPT1A* [41], *ABCG1* [42], and *SREBF* isoforms [43] has previously been correlated with histone modifications in human cells or animal models.

In addition to functional studies of histone marks in known lipid loci, ChIP technology offers the potential to identify novel epigenetic correlates of metabolic phenotypes, although these studies are yet to be implemented on a large scale in humans. In Japanese macaques, in utero exposure to a high-fat calorie-dense maternal diet has been shown to alter histone acetylation patterns, putatively correlated with expression at several genes of interest, e.g. *Npas2* [44]. In humans, disrupted expression of circadian genes such as *NPAS2* has been extensively linked to abnormal metabolic phenotypes, specifically dyslipidemia (reviewed in [45]), yet the underlying mechanisms are not well understood; epigenetic processes such as post-translational histone acetylation warrant future studies as potential mediators.

Any discussion of histone modifications in the context of lipids would be remiss to exclude statins, a broad class of drugs successfully used to lower LDL cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A). In addition to their primary lipid-lowering action, statins also inhibit histone deacetylases (HDACs [46]), notably HDAC2 in atherosclerotic plaques of human coronary arteries, and modulate oxidized LDL-induced expression of inflammatory genes [47]. While these mechanistic insights have generated enthusiasm, particularly in oncology [48], they have yet to be translated into clinical breakthroughs.

RNA-based mechanisms

A number of non-coding RNAs, including small (<200 nucleotides) and long (≥200 nucleotides) non-coding RNAs, can indirectly regulate gene expression via interactions with

canonical epigenetic processes such as histone acetylation/methylation or DNA methylation, as well as directly by targeting mRNA [49]. The majority of research on their role in lipid homeostasis to date has focused on microRNAs (miRNAs), which have been shown to control lipoprotein levels in plasma (summarized in [50]) through several mechanisms. A useful example is the well-studied miR-33a/b, previously discussed in the context of DNA methylation findings. In experimental models, miR-33 isoforms decreased hepatic biogenesis of HDL cholesterol and attenuated cellular cholesterol efflux in an ABCA1 and ABCG1 dependent manner [51]. Additional evidence suggests that miR-33 inhibition promotes regression of atherosclerotic plaques by affecting both lipid transport [52] and inflammation [53]. Similar positive effects on plasma lipid profiles were achieved in nonhuman primates [54], raising the therapeutic relevance of these discoveries. On the other hand, miR-33 knockout mice fed a high-fat diet also exhibited increased total plasma cholesterol, obesity, insulin resistance, and fatty liver, mediated by interactions with hepatic SREBP-1 [25]. These deleterious metabolic effects, however, were not observed with miR-33 inhibition rather than genetic deletion [55]. MiR-33 also has other targets, including *CPT1A* and p53 (reviewed in [56]), although the current understanding of such disparate effects is rather limited. The miR-33 story highlights the translational promise of small regulatory RNAs in dyslipidemia and urges caution in regard to potential pleiotropic effects of any miR-based therapies.

The role of long noncoding RNAs (lncRNAs) in lipid homeostasis is also coming to sharper focus, with multiple *in vitro* studies implicating lncRNAs in hepatic lipid metabolism and adipogenesis (reviewed in [57]). For example, a recent study identified LeXis (liver-expressed LXR-induced sequence), a lncRNA located in close proximity to *Abca1* in the murine genome, as a mediator of the liver X receptor-induced cholesterol efflux and simultaneous decrease in cholesterol synthesis [58]. However, because lncRNAs are not as highly evolutionarily conserved as miRNAs [57], the enthusiasm for their potential therapeutic use must remain tempered until proof of concept from human studies. Overall, RNA-based processes represent an important frontier in epigenetic studies of lipids, and future large-scale human studies are poised to take advantage of shotgun transcriptomics technologies (RNASeq) to further explore these mechanisms.

Conclusion

The epigenetic signature of dyslipidemia is complex, spanning multiple genomic regions, biochemical processes, and phenotypes. The relationship between lipids and epigenetic markers is dynamic and likely bidirectional; while lipid levels pattern methylation of key relevant genes (*CPT1A, ABCG1, SREBF1, DHCR24*), other epigenetic processes (e.g. regulation of gene expression by miR-33) are likely a cause rather than a consequence of altered lipid profiles. The genomic regions implicated in dyslipidemia often harbor more than one type of epigenetic change, as illustrated by the relationship between miR-33 and methylation findings in *ABCG1* and *SREBF1*, and epigenetic effects of each variant can be pleiotropic. Future efforts aiming to realize the nontrivial translational potential of epigenetic findings in dyslipidemia would benefit from heeding the intricate variability of the observed patterns across time, tissue, and physiologic context.

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Table 1

Selected studies of candidate gene and repetitive element methylation and lipid phenotypes.

Repetitive Element Methylation Studies			
Reference	**Population (tissue)**	**N**	**Phenotype(s): associated changes in global methylation**
[59]	European (whole blood)	738	HDL-c: hypomethylation
[60]	European (whole blood)	228	TC, LDL-c, TG, HDL-c: hypomethylation
[61]	Samoan (whole blood)	355	LDL-c, HDL-c: hypomethylation
Candidate Gene Studies			
Reference	**Population (tissue)**	**N**	**Phenotype(s): associated gene**
[62]	Canadian with severe obesity (whole blood, adipose tissue)	73	LDL-c: *LEP, ADIPOQ*
[63]	Chinese children (whole blood)	98	LDL-c, TG, HDL-c: *FAIM2*
[64]	European American (CD4+ T cells)	993	TC, LDL-c: *APOE*
[65]	European children with obesity (whole blood)	85	TG: *IGF2*
[66], [67]	French Canadian with untreated familial hypercholesterolemia (whole blood)	98	LDL-c, HDL-c, TG: *ABCG1, LIPC, PLTP* HDL-c: *ABCA1*
[68]	European American (whole blood)	517	TC, LDL-c: *FABP3*
[69]	Chinese with diabetes and healthy controls (peripheral blood mononuclear cells)	47	TG: *MCP1*
[70]	Canadian women with severe obesity (visceral adipose tissue)	92	HDL-c: *DPP4*

Table 2

Epigenome-wide studies of DNA methylation and lipid phenotypes.

Reference	Array	Population (tissue)	N	Phenotype(s): Top associated CpG (gene)
[11]	Illumina 27K	French Canadian with familial hypercholesterolemia (whole blood)	Discovery: 21 Replication: 70 (untreated) and 178 (treated with lipid-lowering drugs)	HDL-c: cg07189381 (*TNNT1*)
[12]	Illumina 450K	European American (CD4+ T cells)	Discovery: 663 Replication: 331	VLDL and LDL subfraction parameters: cg00574958 (*CPT1A*)
[17]	Illumina 450K	European American (CD4+ T cells)	Discovery: 991 Replication: 2711	VLDL-c and TG: cg00574958 (*CPT1A*)
[15]	Illumina 450K	European (whole blood, adipose tissue, skin)	Discovery (whole blood): 1776 Replication (whole blood): 971 Replication (adipose): 634 Replication (skin): 395	LDL-c: cg22178392 (*TNIP1*), TG and HDL-c: cg06500161 (*ABCG1*)
[26]	Illumina 450K	European American (CD4+ T cells)	443	Change in lipids following fenofibrate treatment: no significant findings
[22]	Reduced Representation Bisulfite Sequencing	European, survivors of the Dutch Hunger Winter and sibling controls (whole blood)	120	LDL-C: differentially methylated region in *CPT1A*
[7]	Illumina 450K	European (whole blood)	3296	LDL-c: cg27168858 (*DHCR24*), TG: cg11024682 (*SREBF1*), cg06500161/ cg27243685 (*ABCG1*) and cg00574958/ cg17058475 (*CPT1A*), HDL-c: cg06500161/ cg27243685 (*ABCG1*)