Effects of lipids on DNA methylation and gene expression of immune cells implicated in atherosclerosis

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Chapter 2

The multifaceted interplay between lipids and epigenetics

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Abstract

Purpose of review

The interplay between lipids and epigenetic mechanisms has recently gained increased interest because of its relevance for common diseases and most notably atherosclerosis. This review discusses recent advances in unravelling this interplay with a particular focus on promising approaches and methods that will be able to establish causal relationships.

Recent findings

Complementary approaches uncovered close links between circulating lipids and epigenetic mechanisms at multiple levels. A characterization of lipid-associated genetic variants suggests that these variants exert their influence on lipid levels through epigenetic changes in the liver. Moreover, exposure of monocytes to lipids persistently alters their epigenetic makeup resulting in more proinflammatory cells. Hence, epigenetic changes can both impact on and be induced by lipids.

Summary

It is the combined application of technological advances to probe epigenetic modifications at a genome-wide scale and methodological advances aimed at causal inference (including Mendelian randomization and integrative genomics) that will elucidate the interplay between circulating lipids and epigenetics. Understanding its role in the development of atherosclerosis holds the promise of identifying a new category of therapeutic targets, since epigenetic changes are amenable to reversal.

Introduction

The development of atherosclerosis, the underlying cause of cardiovascular diseases, including myocardial infarction and stroke, is driven by interactions between blood lipids and circulating immune cells [1]. Recent findings highlight epigenetic mechanisms as an important mediator of these interactions. Elucidating the precise role of epigenetic mechanisms is expected to reveal molecular pathways in the development of atherosclerosis that are in principle modifiable [2]. A main challenge in the field is the fact that the relationship between lipids and epigenetics is multifaceted: lipids can induce epigenetic changes or vice versa, and both mechanisms may promote atherosclerosis. This review will describe current knowledge of the interplay between lipids and epigenetic mechanisms focusing on studies performed in human populations or in human-derived cells. Moreover, methodological advances will be discussed that may help in teasing apart cause and consequence, including in Mendelian randomization and integrative genomics.

Key points

- The interplay between lipids and epigenetic mechanisms may affect common diseases including atherosclerosis.

- Emerging evidence shows that circulating lipids can not only influence the epigenome, resulting in an epigenetically primed cell, but also support the reverse relationship, in which epigenomic changes result in alterations of lipid metabolism.

- Epigenetics is intertwined with genetic variation and gene expression and it is the interplay between these levels of genomic information that should be studies to understand how the differences in disease risk between individuals are shaped.

Lipid-associated genetic variants affect the epigenome

The majority of variation in circulating lipid levels between individuals is explained by genetic factors [3]. For rare Mendelian dyslipidaemias such as familial hypercholesterolaemia, many severe mutations have been identified that abolish protein function and that together explain the majority of patients [4]. To pinpoint the specific sequence variants underlying the role of genetic factors in the general population, genome-wide association studies (GWASs) can be performed in which hundreds of thousands up to millions of common genetic variants are evaluated for their association with blood lipids levels. GWASs for lipid levels have been particularly successful. The most recent GWAS on lipids included 188,577 individuals and identified 57, 69, and 40 genetic variants associated with levels of LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides, respectively [5]. Together, these lipid-associated variants explain 10–15% of the variation in these lipid levels in the general population [5]. However, in contrast to the rare mutations underlying dyslipidaemias, only a small minority of 7% of the lipid-associated variants results in an amino acid change (nonsynonymous variant), whereas only an additional 9% of variants maps to exons without changing an amino acid (synonymous variant) or maps to an untranslated region. Hence, the very large majority of lipid-associated variants will exert its effects by changing the regulation of gene expression. Epigenetic mechanisms play an essential role in the regulation of gene expression. These mechanisms include the methylation of cytosine bases in the DNA (particularly in CpG dinucleotides) and a multitude of modifications of the histones around which the DNA is wrapped [6]. Together they control the accessibility of the DNA to the transcriptional machinery and govern the stable regulation of transcription. As such, epigenetic regulation is involved in many cellular processes, including cell differentiation and cell activation [7]. Hence, the essence of epigenetic marks is their cell and tissue specificity [8▪▪, 9].

Key to the current progress in charting the impact of noncoding sequence variants through epigenetic mechanisms are large-scale epigenome projects in which cell type-specific, whole-genome reference maps of histone modifications, and DNA methylation are created [8▪▪, 10]. The combination of histone modifications that is present at a genomic region is an informative indicator of its biological role. For example, the presence of the activating histone mark histone-3 lysine-4 trimethylation (H3K4me3) in a cell type signals an active transcription start site,

whereas histone-3 lysine-4 monomethylation (H3K4me1) marks the presence of an enhancer element. Similarly, repressed regions are marked by histone modifications, including the Polycomb repression mark histone-3 lysine-27 trimethylation. A recent study characterized the overlap of histone modification in 127 different cell types with lipid-associated genetic variants identified through GWAS [8▪▪]. Interestingly, the genetic variants were highly enriched in genomic regions harbouring an active histone mark specifically in the liver (i.e. H3K4me3, and histone-3 lysine-7 acetylation, H3K27ac), the tissue that is responsible for the synthesis and clearance of lipids, whereas such enrichment was absent for other tissues. These data show that lipid-associated variants colocalize with genomic regions under active epigenetic control in the liver and suggest that the effect of the variants coincides with changes in the local epigenetic state. Indeed, a recent study demonstrated that genetic variants can modify local histone marks and that these changes are often associated with gene expression activity, either directly or through altered transcription factor binding [11].

A similar approach can be applied to genome-wide DNA methylation data. DNA methylation has multiple functions and is an informative marker of transcriptional regulation [12]. Genetic factors have a major impact on DNA methylation variation [13, 14] and genome-wide studies identified specific sequence variants that affect local DNA methylation variation, called methylation quantitative trait loci (meQTLs) [15,16]. A recent GWAS showed that genetic variants associated with blood pressure are meQTLs more frequently than expected by chance [17] indicating that the downstream effects of the genetic variants is either mediated directly through DNA methylation or through a process that is marked by DNA methylation changes. To test whether genetic variants associated with lipid levels [5] also are enriched for meQTLs, we performed a look-up in a recently established catalogue of meQTLs based on 3840 whole blood samples [18]. We found that 30 (out of 57), 31 (out of 69), and 16 (out of 40) of the genetic variants associated with LDL-C, HDL-C, and triglycerides, respectively, are meQTLs (Table 1). An additional look-up in meQTL sets discovered in liver (n = 161) and adipose tissue (n = 71) [19▪] resulted in an even greater overlap of lipid-associated variants with meQTLs in the liver despite the much smaller sample size (and consequently lower statistical power) than the study in blood (Table 1). As liver obviously plays a more important role in determining circulating lipid levels than blood and adipose tissue, this observation suggests that the effects of noncoding,

lipid-associated genetic variants may be mediated by a modification of the epigenome.

Table 1: Genetic variants associated with lipid levels often affect DNA methylation.

		Number of methylation QTLs			
Lipid	GWAS variants	Blood (n = 3840)	Liver (n = 161)	SAT (n = 71)	VAT (n = 71)
LDL-C	57	30 (53%)	28 (49%)	8 (14%)	9 (16%)
HDL-C	69	31 (45%)	38 (55%)	7 (10%)	10 (14%)
TG	40	16 (40%)	26 (65%)	6 (15%)	7 (18%)
Total	136	63 (63%)	72 (53%)	16 (12%)	20 (15%)

GWAS, genome-wide association study; HDL-C, HDL cholesterol; LDL-C, LDL-cholesterol; QTLs, quantitative trait loci; SAT, subcutaneous adipose tissue; TG, triglycerides; VAT, visceral adipose tissue.

The overlap between lipid-associated variants and methylation quantitative trait loci was higher for liver [19▪] than for whole blood [18] and adipose tissue [19▪] in line with the key role of the liver in secretion and clearance of lipids in and from circulation.

Lipid-induced changes in the epigenome

Although the epigenome plays a role in mediating the effects of genetic variants, the main push for epigenetics research has been the notion that the epigenome can respond to intrinsic and extrinsic signals [20]. For example, external environmental factors such as smoking [21] and malnutrition [22] are associated with persistent changes in DNA methylation. Blood lipids are an important component of the environment of immune cells in the circulation. As the interaction between lipids and immune cells drives the development of atherosclerosis, a recent study [23▪▪] investigated whether these cells can be epigenetically primed by exposure to oxidized LDL (oxLDL), the key form of LDL particles in the formation of atherosclerosis [24]. Purified monocytes were exposed to oxLDL for 24h *in vitro* and subsequently cultured under standard conditions for 6 days to allow the monocytes to differentiate into macrophages. Pre-exposure with oxLDL led to macrophages with a proatherogenic cytokine and chemokine profile comprising an upregulation inflammatory gene. Moreover, the expression of lipid metabolism genes such as scavenger receptors

(*MSR1* and *SCARB1*) and lipid transporters (*ABCA1* and *ABCG1*) was altered. Region-specific chromatin immune precipitation sequencing showed that these expression changes were because of the deposition of the active chromatin mark H3K4me3 near proatherogenic genes in response to oxLDL pre-exposure. Of interest, blocking methyltransferase activity abolished the pre-exposure response supporting a causal role of epigenetic remodelling. This study provided compelling evidence for lipid-induced epigenetic priming of immune cells leading to long-term changes of the cellular phenotype. It will, however, be key to translate these in-vitro findings to the in-vivo situation, especially since the copper oxLDL used in this study does not represent physiological oxLDL [23▪▪], and, subsequently, to establish whether epigenetic priming plays a role in the cause of atherosclerosis.

Approaches to study epigenetic priming in vivo are provided by clinical trials evaluating the impact of lipid-modifying medication. So far, a single study was performed that compared genome-wide DNA methylation measured in whole blood (n = 443) before and after a 3-week treatment with the lipid-modifying drug fenofibrate and did not identify drug-induced epigenetic differences [25]. Fenofibrate, however, only moderately altered lipid levels in this study (−15% LDL-C, +7% HDL-C, and −35% triglycerides). Combined with a relatively short follow-up period, this could explain why no differences were observed. To prove or disprove the occurrence of lipid-induced epigenetic priming using such approaches, it will be fruitful to design studies in such a way that they are able to test a specific hypothesis that resulted from in-vitro studies. This implies purifying the same cell type as was studied *in vitro*, evaluating the effects of drugs targeting the relevant lipid level, and focusing the analysis on genomic regions undergoing epigenetic changes *in vitro* (instead of performing all possible tests at the expense of statistical power and the interpretability of outcomes). Conversely, *in vitro* studies can be designed in such a way that the outcomes can be validated in vivo using available clinical trials.

Interplay between lipids and the epigenome: epidemiological approaches

Analogous to GWAS, epigenome-wide association studies (EWASs) are increasingly being performed [26]. In an EWAS, methylation levels are measured of hundreds of thousands of CpGs distributed across the genome in, currently, hundreds to thousands of individuals. Subsequently the methylation level of every individual CpG

is evaluated for an association with an outcome of interest. EWASs have commonly used the Illumina 450k array [27] to measure genome-wide DNA methylation because of its favourable price/content ratio. It interrogates methylation level of approximately 480,000 CpGs and, although primarily focussing on the coverage of promoters and CpG islands, it also targets many other types of regulatory sequences [8▪▪]. More recently, the Illumina EPIC 850k array was launched which measures about twice as many CpGs and focusses on covering enhancers on top of the original content of the 450k array [28]. Nevertheless, even the new array covers only ~3% of the 28 million CpGs in the human genome. Despite similarities in terminology, EWASs are exceedingly more complicated to execute properly than GWASs since, in contrast to the inert DNA sequence, DNA methylation is dynamic, cell and tissue specific, variable over the life course, and subject to confounding and other limitations common in observational epidemiology. This topic has been discussed in detail by other reviews [29, 30].

A recent EWAS of 649 metabolic traits in whole blood samples (n = 1814) identified associations of multiple lipids (including cholesterol, sphingolipids, and glycerophospholipids) and lipoprotein particles (including LDL, HDL, and VLDL particles) with the methylation level of CpGs in or near the genes *DHCR24*, *TXNIP*, *SLC25A22*, *CPT1A*, *MYO5C*, and *ABCG1* [31▪]. A second EWAS in CD4+ T cells (n = 663) observed associations between LDL and VLDL levels and CpGs in *CPT1A* [32], which was later replicated in blood (n = 526) [33]. The most recent EWAS in whole blood (n = 1776) observed associations between DNA methylation and triglycerides for CpGs mapping to the genes *CPT1A*, *ABCG1*, *SREBF1* and *SCD*, between DNA methylation and HDL-C for a CpG in *ABCG1*, and between DNA methylation and LDL-C for a CpG in *TNIP1* [34]. Several associations were subsequently replicated in adipose tissue (n = 634) and skin (n = 395), indicating that associations are not necessarily tissue specific [34]. Most of these genes (including ABCG1 which was also identified as differentially expressed after oxLDL exposure in the in-vitro study previously discussed [23▪▪]) have an important function in lipid metabolism, supporting a regulatory role of epigenetic mechanisms. Interestingly, EWASs of BMI [35, 36], waist circumference [35], type 2 diabetes [37], insulin, and insulin resistance [38] found several of the same differentially methylated CpG. As these traits are related, some overlap is expected. However, it is striking that the overlapping CpG sites are located in lipid metabolism genes. As all EWASs so far were performed with the Illumina 450k array, current findings likely represent the tip of the iceberg because of its sparse coverage.

A major limitation in the interpretation of EWASs is that they cannot infer causality, and it thus remains unclear whether lipids influence DNA methylation (compatible with the concept of epigenetic priming) or DNA methylation causes differences in lipid levels. To infer the direction of the relationships, genetic variants can be used as causal anchor because of their invariable nature. This idea has been formalized as Mendelian randomization [39, 40▪▪], in which genetic variants associated with a specific trait are used to construct a genetic predictor that subsequently is used to estimate the effect of this trait on other traits. Mendelian randomization was specifically put forward as a tool for causal inference in DNA methylation studies [39, 40▪▪] and was recently applied to show that a DNA methylation difference near *HIF3A* was the consequence of interindividual variation in BMI using a genetic predictor based on BMI-associated genetic variants [41]. For the study of lipids, Mendelian randomization may be particularly promising, since lipid-associated genetic variants provide robust predictors of LDL-C, HDL-C, and triglyceride levels. Accordingly, Mendelian randomization has been used to interrogate the causal relationship between elevated lipid levels and the risk of type 2 diabetes [42] and coronary artery disease [43]. Future studies can use these predictors to estimate the effect of lipid levels on DNA methylation and the reverse situation, where DNA methylation influences lipid levels can be estimated using meQTLs (Figure 1). A limitation of Mendelian randomization, however, is that even larger sample sizes are needed than in regular EWASs, since the predictors explain a relatively small proportion of the variation in their respective trait (10–15% in the case of blood lipid levels) [5]. This will be a particular problem when studying tissues that are difficult to obtain in humans but are crucial in lipid metabolism such as liver samples. Moreover, Mendelian randomization requires careful consideration of assumptions, including the absence of direct effects of lipid-associated variants on DNA methylation and absence of effects of genetic variants on multiple traits (pleiotropic effects). Further methodological developments, such as Egger regression, which can provide valid estimations of causal effects in the presence of pleiotropy, will be instrumental in this respect [44].

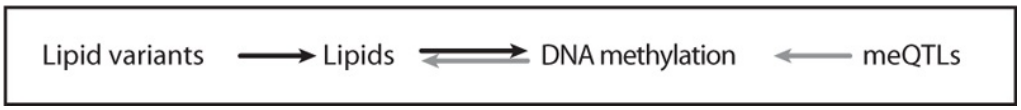

Figure 1: In Mendelian randomization lipid-associated genetic variants (identified in genome-wide association studies) can be used to estimate the effect of lipids on DNA methylation (black) and methylation quantitative trait loci can be used to estimate the effect of DNA methylation on lipid levels (grey).

Beyond the epigenome: integrative genomics

It has become increasingly clear that the epigenome cannot be studied in isolation [30]. It is intertwined with genetic variation and gene expression and it is the interplay between these levels of genomic information that shapes the differences in disease risk between individuals. Therefore, an integrative genomics approach will be an important way forward to understand the relationship between circulating lipids and the epigenome, and its role in the development of atherosclerosis.

A straightforward approach to data integration is to calculate associations between individual measurements of different molecular layers. As described earlier, genetic variants can be associated with local DNA methylation levels to uncover methylation QTLs [18]. Similarly, epigenetic differences should be associated with gene expression to link it to potential downstream effects. For example, the lipid-associated CpGs identified in EWASs were associated with the expression of *CPT1A* (involved in catabolism of lipids), *ABCG1* (involved in cellular export of lipids), and *SREBF1* (involved in lipid homeostasis) [34], which further corroborates that the observed DNA methylation differences reflect an altered regulation of lipid metabolism.

An alternative approach to the integration of molecular data is to first cluster the measurements within each layer and then test these clusters for associations across layers. A recent study identified associations between clusters of genome-wide measurements of multiple histone modifications, transcriptome, and DNAse I sensitivity (marking active regulatory DNA) for monocytes, macrophages, and macrophages pre-exposed to lipopolysaccharide [45]. Further analysis of these clusters showed that relevant pathways were epigenetically activated in each cell type, for example sugar binding in macrophages pre-exposed to lipopolysaccharide. To extend the concepts from this study to lipid-induced epigenetic priming, the same

design may be adopted using cells from liver, adipose tissue, or blood (including monocytes or T cells that play a role in atherosclerosis) that are isolated either from healthy individuals and then primed with lipids *in vitro* or from hyperlipidaemic individuals, whose cells have been primed by high lipid levels in vivo. Such *ex vivo* approaches may also be used to explore the ability of epigenetic drugs [2] to reverse lipid-induced epigenetic priming.

A third approach that is applied to multiple layers of molecular data comprises the construction of networks of correlations between and across the different layers simultaneously. In a recent study, such a network was constructed using measurements of 440 metabolic traits and whole-genome transcription data measured in blood [46]. Specific parts of this network were associated with triglycerides, LDL-C, and HDL-C and many of the nodes in the network corresponded to genes involved in lipid metabolism.

Although the multi-omics approaches discussed so far are limited to describing correlations and are generally limited to linking two levels only, a recent study tried to establish causal relationships between more than two layers of genomic information [47]. To estimate causal relationships, a Bayesian network was used (validated with the omnibus test for causal inference [48]) to test whether a genetic variant affected DNA methylation and in turn affected transcription or, conversely, whether a genetic variant affects transcription and in turn affects DNA methylation. The study revealed examples of both situations and showed that this interplay is often associated with expression of local transcription factors, demonstrating multidirectional interactions between different omics layers.

A promising way forward is to extend the Mendelian randomization framework to incorporate multiple data layers, hence using genetic variants associated with lipid levels, DNA methylation (methylation QTLs), and transcription (expression QTLs) (Figure 2). Not only will this approach be able to infer causal relationships but also is robust against spurious associations because of unmeasured confounding. Here, it will be crucial to separate expression from methylation QTL effects of a genetic variant, which may not always be possible for local effects. In addition, approaches that combine recent development to predict tissue-specific gene expression (and putatively DNA methylation) using genome-wide genetic profiles [49▪] and extensions of Mendelian randomization robust to pleiotropic effect should be used [44].

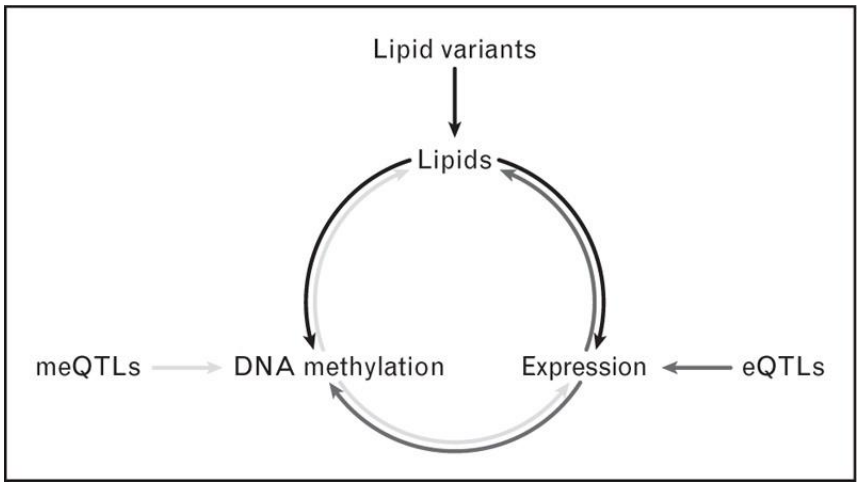

Figure 2: In an integrative genomics approach to Mendelian randomization, lipid-associated genetic variants (identified in genome-wide association studies) are used to infer the effect of lipids on DNA methylation and gene expression (black), methylation quantitative trait loci to infer the effect of DNA methylation on lipid levels and gene expression (light grey), and expression quantitative trait loci to infer the effect of gene expression on lipid levels and DNA methylation (dark grey).

Conclusion

The interplay between lipids and epigenetics in humans is bidirectional, tissue specific, and connected to other omics layers. Emerging evidence supports the concept that lipids can not only influence the epigenome, resulting in an epigenetically primed cell, but also vice versa in which epigenomic changes result in alterations in lipid metabolism. Integrating genome-wide epigenetic data with other layers of molecular data, such as genetics, transcriptomics, and metabolomics data, accompanied by relevant statistical methods, such as Mendelian randomization and other causal inference approaches, will enhance our understanding of the bidirectional interaction between lipids and cellular functions. As epigenetic changes are potentially reversible, these developments hold the promise of discovering new therapeutic targets for lipid-related diseases such as atherosclerosis [2].

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Conflicts of interest

There are no conflicts of interest.

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▪ of special interest

▪▪ of outstanding interest

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