DNA methylation and epigenetics: exploring the terra incognita of the atherosclerotic landscape

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This editorial refers to ‘Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster’¹, by E. Aavik et al., on page 993.

Despite the fact that DNA was discovered already > 70 years ago and the sequencing of the human genome was completed in 2003, many questions still remain unanswered as far as the translation of genotype to phenotype is concerned. In the past decades, several long-standing dogmas in genetics have been revisited so that we now appreciate that genes may not always be expressed in every cell type, DNA may not always be transcribed into RNA, and RNA may not always be further translated into protein. The complexity in understanding the ultimate phenotype engendered in the genetic code could be partly explained by the emerging field of epigenetics. Originally defined by C.H. Waddington as ‘the causal interactions between genes and their products which bring the phenotype into being’, this novel area of investigation aims to elucidate heritable changes that are not a mere consequence of modifications in the DNA sequence. Indeed, a major role of epigenetics may occur at the level of chromatin structure and organization, regulating the accessibility to DNA by various cellular machineries and therefore affecting gene expression. The principal epigenetic modification of DNA is mediated by the covalent attachment of a methyl group to the cytosine in cytosine–guanosine (CpG) dinucleotides.¹ Methylation of CpG exerts its effects in a context-specific manner, repressing gene expression when located in gene promoter or regulatory regions, but promoting gene expression when present in the gene body. As such, DNA methylation plays a key role in many biological processes including genome organization, imprinting, X chromosome inactivation, and the regulation of gene expression and RNA splicing. Notably, methylation of CpG is a highly dynamic process and reveals distinct patterns under particular conditions, e.g. ageing or disease, thus moving into the focus of research in haemat-oncology, neuroscience, and diabetes.²

Embarking on the expedition from genetics to epigenetics

Although a genetic predisposition to atherosclerosis has been widely recognized as a risk factor, extensive genome-wide association studies have recently revealed that only 10.6% of cases could be explained by a heritable genetic component, thus implying a role for other factors acting beyond gene sequence.³ In fact, recent studies have successfully linked cardiovascular risk factors such as chronic inflammation or diabetes and familial hypercholesterolaemia to a modification of the DNA methylation status. Moreover, the expression of DNMT1 as a pivotal enzyme in DNA methylation is regulated in endothelium exposed to atherogenic flow conditions and LDLs, where it controls epigenetic modulation of gene expression and can foster endothelial inflammation and development of atherosclerosis.⁴,⁵ Finally, differential methylation in specific loci (including LINE, CETP, LPL, FOXP3, and others) has been identified as a potential biomarker for atherosclerosis.⁶ To date, however, few studies have investigated the global status of DNA methylation in human atherosclerosis and few data are available regarding the gene compartments affected by this process and the functional relevance in terms of expression of individual genes.⁶–⁸ In this context, the technological advances such as next-generation sequencing now allow for a more accurate approach to investigate DNA methylation and have been effectively applied for identifying significant DNA methylation changes in several human diseases.²

Aavik and colleagues now highlight new findings on DNA methylation patterns in human femoral atherosclerotic plaques compared with healthy mammary arteries.⁹ Interestingly, the authors report that changes in methylation status are a frequent phenomenon in atherosclerotic plaques and outline several genes which are differentially

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methylated in at least one of the gene compartments, and de facto the most common alteration was hypomethylation. When only considering the promoter region, where methylation is known to exert relevant effects on repressing gene expression, hypomethylation was found in ~84% of the cases. Using gene ontology analysis, the authors then demonstrate mainly keratins and keratin-associated proteins to be preferentially included in this group. Clearly, hypomethylation was also the prominent methylation alteration in gene bodies (both introns and exons), and gene ontology analysis identified ATP-binding proteins and proteins associated with the cytoskeleton and chromatin homeostasis as the most affected cluster of genes. The functional relevance of these changes in methylation was confirmed by the observation that the majority (~82%) of the hypomethylated genes exhibited an increase in transcription of mRNA. Conversely, hypermethylation was found only in a minority of cases and their correlation with down-regulation of gene expression was rarely confirmed; indeed, only a few genes (11 out of 271) found to be hypermethylated in the promoter region were effectively down-regulated at the mRNA level, whereas hypermethylation in the gene body was preferentially associated with transcriptional up-regulation. Interestingly, gene ontology analysis identified ATP-binding and cytoskeleton proteins to be the most affected also among the hypermethylated genes. The authors then focused on specific genomic loci, in particular investigating the DNA methylation status at locus 9p21. This locus represents the most replicated genetic factor for coronary artery disease and myocardial infarction identified by genome-wide association studies. Among the genes encoded in this region, CDKN2A, CDKN2B, and MTAP have been implicated in the biology of vascular smooth cells, showing a robust expression in human atherosclerosis but no association with the 9p21 genotype.10 In this context, the long non-coding RNA CDKN2BAS (also known as ANRIL) is currently considered as the most probable candidate for linking the 9p21 locus to atherosclerosis, and the finding that differences in methylation of CDKN2BAS, with exon 7 found to be hypomethylated and exon 8 being hypermethylated, could support at least a partial role for DNA methylation in mediating such a detrimental effect.

**Extending the borders: interplay of post-transcriptional modulators**

One of the most interesting findings of the study is surely the observation of several changes in the methylation status of promoter of genes encoding microRNAs (miRNAs). MiRNAs are small non-coding RNAs that act as key post-transcriptional modulators of gene expression.11 Over 1000 miRNAs have been identified in humans, and they are globally able to regulate the expression of ~60% of protein-coding genes. Knowledge concerning the contribution of miRNAs to the development and evolution of atherosclerosis has steadily grown within the last years, and their expression in human atherosclerotic plaques has been assessed.11–14 Interestingly, the authors found that > 140 miRNA sites were subject to hypomethylation in atherosclerotic plaques, including some already linked with atherosclerosis (e.g. miR-10b, miR-27b, and miR-758, amongst others) in different experimental settings (Figure 1). Remarkably, the promoters showing the highest level of hypomethylation were located in the chromatin locus 14q32. This region hosts the largest cluster of miRNAs currently identified in the human genome, encoding 54 miRNAs.15 Among the miRNAs encoded in this area, a four- to eight-fold up-regulation of miR-127,

![Figure 1](image-url)  
**Figure 1** DNA methylation and atherosclerosis. Cardiovascular risk factors have been associated with changes in DNA methylation status, and femoral atherosclerotic plaques show deep changes in DNA methylation. Atherosclerotic plaques display hypomethylation involving various microRNAs sites, many of which may play a potential role in the pathogenesis of atherosclerosis, as indicated. The elucidation of detailed mechanisms and options to revert safely or to modify the methylation status of respective microRNAs in atherosclerosis should be meticulously scrutinized in future studies to pave the way for possible therapeutic developments.
-136, -410, -431, -432, and -433 was confirmed in atherosclerotic plaques. Globally, these miRNAs are predicted to target several genes whose expression was found to be down-regulated in the microarray analysis. Of note, the expression of miR-127 has already been described in human atherosclerotic plaques, where it correlates with a clinically vulnerable phenotype. Moreover, the identification of 14q32 as one of the loci most subjected to changes in DNA methylation and the extensive changes found in this region regarding other post-transcriptional modulators, namely miRNAs. This gives rise to a new perspective and adds a layer of complexity in terms of ultimately modulating gene expression in human diseases, and namely in atherosclerosis.

**Towards uncharted territories: future perspectives and possibilities**

Albeit that it is just a pioneering and exploratory expedition, the study by Aavik et al. is striking because it underscores the crucial importance of the genome-wide methylation status of human atherosclerotic plaques and integrates the findings with an extensive analysis of gene expression. Indeed, we are just beginning to enter the first stages towards exploring the role of epigenetics in the 90% of the terra incognita affecting heritability in the overall landscape of atherosclerosis. While the current study undoubtedly represents an at-tractive point of departure providing possible directions, future expeditions will be required to map and understand fully the role of epigenetics in human atherosclerosis. In particular, these should aim to explore the methylation status in different arterial districts (e.g. femoral plaques actually reflect an advanced state of atherosclerosis and usually do not display a vulnerable phenotype), to identify the cell-specific methylation patterns, to clarify the mechanisms underlying these changes through in vitro and in vivo experiments, and to investigate the role of other epigenetic tags (i.e. histone deacetylation). The far horizon of these expeditions could certainly open up new avenues and approaches for preventing and treating atherosclerosis and its complications.

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**References**