# REVIEW ARTICLE

# Clinical applications of epigenetics in cardiovascular disease: the long road ahead

**STELLA ASLIBEKYAN, STEVEN A. CLAAS, and DONNA K. ARNETT**

**BIRMINGHAM, ALA**

**Epigenetic processes, defined as heritable changes in gene expression that occur without changes to the DNA sequence, have emerged as a promising area of cardiovascular disease research. Epigenetic information transcends that of the genotype alone and provides for an integrated etiologic picture of cardiovascular disease pathogenesis because of the interaction of the epigenome with the environment. Epigenetic biomarkers, which include DNA methylation, histone modifications, and RNA-based mechanisms, are both modifiable and cell-type specific, which makes them not only responsive to the environment, but also an attractive target for drug development. However, the enthusiasm surrounding possible applications of cardiovascular epigenetics currently outpaces available evidence. In this review, the authors synthesize the evidence linking epigenetic changes with cardiovascular disease, emphasizing the gap between the translational potential and the clinical reality of cardiovascular epigenetics. (Translational Research 2014;■:1–11)**

**Abbreviations:** apoA-I = apolipoprotein A-I; ASSURE = ApoA1 Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation; BET = bromodomain and extraterminal; CpG = cytosine-phosphate-guanine; CVD = cardiovascular disease; HDAC = histone deacetylase; HDL = high-density lipoprotein; *LINE-1* = long interspersed nucleotide element 1; SUSTAIN = Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation

Despite its initial promise, the clinical utility of genetic markers for prediction and prevention of heart disease has proved to be limited.[1] After this translational disappointment, the attention of the cardiovascular research community turned to epigenetics as the new frontier for risk stratification, prevention, and treatment. Broadly defined, epigenetics is the study of heritable changes in gene expression that are not coded in the DNA sequence itself.[2] Epigenetic variation falls into 3 interconnected categories: DNA methylation, RNA-based mechanisms including microRNAs and noncoding RNAs, and posttranslational histone modifications.[3] (Throughout this review, we use "epigenetic" to refer to variations in these 3 categories and "genetic" to refer to polymorphisms in the DNA sequence.)

Although DNA methylation is the most common epigenetic modification in the mammalian genome,[4] a growing body of evidence suggests all 3 types of epigenetic changes are involved in the pathogenesis of cardiovascular disease (CVD).[5]

The field of CVD epigenetics is growing rapidly,[6] although the current enthusiasm about its clinical applications far exceeds available evidence. In 2013, 353 of

From the Department of Epidemiology, University of Alabama at Birmingham, Birmingham, Ala.

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Reprint requests: Stella Aslibekyan, PhD, University of Alabama, 1665 University Boulevard, RPHB 230J, Birmingham, AL 35294; e-mail: saslibek@uab.edu.

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the 8026 manuscripts published in the field of epigenetics (4.4%) were related to CVD. However, of those, only 125 reported findings in humans, and of those 125, 66 (52.8%) were reviews rather than original research, and only 4 (0.6%) were clinical trials or population-level studies. Therefore, the need for more translational studies that would evaluate specific epigenetic modifications as both prognostic markers and therapeutic targets remains pressing. The clinical potential for epigenetic markers in CVD is underscored by several factors—chief among them: (1) animal and *in vitro* model evidence linking epigenetic changes to CVD pathways such as atherosclerosis, (2) increased availability of epigenetic analysis technologies at a decreasing cost, and (3) translational success of epigenetics in other chronic disease settings, most notably cancer. The study of epigenetics is also appealing conceptually because processes such as DNA methylation and histone modifications link the genotype and the environment, helping elucidate the mechanisms underlying CVD.

However, the very nature of epigenetic processes makes their study and their interpretation difficult for both researchers and clinicians. Unlike genetic variation, epigenetic changes are cell-type specific, reversible, and susceptible to both inherited and environmental influences.[7] On the one hand, these characteristics describe attractive targets for interventions; on the other hand, the relevance of epigenetic biomarkers as definitive tools for diagnosis or risk stratification is thoroughly confounded by other variables such as age, genotype, and lifestyle—particularly diet and smoking. Unlike studies of genetic markers, epigenetics research must address the problem of reverse causality; just as epigenetic processes may underlie CVD pathogenesis, there is evidence that subclinical or clinical disease influences epigenetic variation.[6] The causality discussion is complicated further by evidence suggesting that epigenetic changes may be secondary to other disease processes (eg, inflammation[8]), and thus represent an epiphenomenon rather than a genuine disease mechanism.[9] Another challenge to clinical applications is posed by tissue specificity; CVD-relevant epigenetic changes may occur in hard-to-access tissues (eg, the myocardium) and may not be reflected in blood, which is used commonly as a diagnostic tissue. Furthermore, DNA methylation patterns can change rapidly and are reversible, so the optimal timing of the measurement relative to disease onset remains unclear. Last, there is the practical issue of storing and interpreting epigenetic data as part of the patient's medical record, which has become more commonplace in oncology but has yet to translate to the CVD context.

Despite these challenges, in recent years a number of epigenetic tags have emerged as promising biomarkers for CVD. Because most studies of RNA-based epigenetic changes and histone modifications are currently limited to animal and *in vitro* models, the following review of potential clinical applications of epigenetic data focuses on DNA methylation. DNA methylation is a covalent chemical modification of DNA that typically involves adding a methyl group to cytosine residues at cytosine-phosphate-guanine (CpG) nucleotides, although non-CpG methylation can also occur.[10] DNA methylation plays a role in mammalian development, transcription, chromatin structure, cellular homeostasis, genomic imprinting, and disease pathogenesis.[11] Commonly, a genomic region with methylated DNA becomes inaccessible to transcriptional machinery and, as a result, gene expression is suppressed.[12] There exists crosstalk between DNA methylation and other epigenetic modifications, notably histone acetylation, which can contribute to aberrant gene regulation and disease.[13] Methylation and the resulting expression changes are usually stable and heritable during mitosis, although stochastic events or environmental factors can alter methylation patterns throughout a lifetime.[14]

## GLOBAL METHYLATION STUDIES

The first studies of epigenetic biomarkers in the context of CVD focused on global DNA methylation, mostly because of the prominent role that homocysteine, an independent vascular disease risk factor, plays in the methylation process. Global DNA methylation refers to a pattern unique to humans and other vertebrates, in whom genomes are heavily methylated in most cell types, and developmental stages and genomewide hypomethylation is often associated with the risk of disease.[15,16] Several human and animal studies have linked increased plasma homocysteine with decreased global methylation, likely occurring as a result of the accumulation of S-adenosyl homocysteine, which in turn inhibits transmethylation reactions.[17] However, the evidence linking global methylation patterns, usually measured in blood cells, with cardiovascular outcomes remains conflicting and comes mostly from cross-sectional studies, limiting causal inference. Although some studies showed that increased homocysteine and decreased global DNA methylation were associated with vascular disease,[18] others reported associations of coronary heart disease with elevated homocysteine but increased global DNA methylation[19] (see Table I[18-40] for a summary of epigenetic markers noted in this review). A subsequent study failed to demonstrate a correlation between global DNA methylation and plasma homocysteine or folate, suggesting that nonfolate-related mechanisms such as