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

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Abstract

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, we synthesize the evidence linking epigenetic changes with cardiovascular disease, emphasizing the gap between the translational potential and the clinical reality of cardiovascular epigenetics.

Despite its initial promise, the clinical utility of genetic markers for prediction and prevention of heart disease has proven to be limited.[1] Following this translational disappointment, the attention of the cardiovascular research community has 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 three interconnected categories: DNA methylation, RNA-based mechanisms including microRNAs and non-coding RNAs, and post-translational histone modifications[1].[3] Although DNA methylation is the most common epigenetic modification in the mammalian genome,[4] a growing body of evidence suggests all three types of epigenetic changes are involved in the pathogenesis of cardiovascular disease (CVD).[5]

The field of CVD epigenetics is rapidly growing,[6] although the current enthusiasm about its clinical applications far exceeds available evidence. In 2013, 353 of the 8026 manuscripts

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[1]Throughout this review, we use “epigenetic” to refer to variation in these three categories and “genetic” to refer to polymorphisms in the DNA sequence.

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 conceptually appealing 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 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 further complicated by evidence suggesting that epigenetic changes may be secondary to other disease processes, e.g. 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, e.g. the myocardium, and not be reflected in blood, which is commonly used 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. Finally, 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 will focus 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 cross-talk 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 generally stable and heritable during mitosis, although stochastic events or environmental factors can alter methylation patterns throughout 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 genome-wide hypomethylation is often associated with the risk of disease.[1516] Several human and animal studies have linked increased plasma homocysteine with decreased global methylation, likely occurring due to 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. While some studies showed that increased homocysteine and decreased global DNA methylation was associated with vascular disease,[18] others reported associations of coronary heart disease with elevated homocysteine but increased global DNA methylation.[19] (See Table 1 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 non-folate related mechanisms such as systemic inflammation may lead to increased global DNA methylation in the setting of CVD.[42] Consistent with this paradigm, an analysis of methylation patterns in dialysis patients demonstrated a relationship between global DNA hypermethylation with systemic inflammation and increased mortality from chronic kidney disease.[20]

Global DNA methylation studies often interrogate repetitive sequences found across the genome, e.g. long interspersed nucleotide element-1 (*LINE*)*-1* and Alu elements. These DNA segments are generally hypermethylated and, as a result, not transcribed, yet they are abundant; for example, *LINE-1* retrotransposon sequences account for approximately 17% of the human genome.[43] Therefore, quantifying DNA methylation in repetitive elements provides a useful picture of global genomic methylation[44] and may indicate susceptibility to disease. Data from male participants of the VA Normative Aging Study showed that hypermethylation of the Alu repetitive element exacerbated the effect of environmental pollution on a range of systemic inflammation markers like C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[21] In the same cohort, *LINE-1* element hypomethylation in blood DNA was associated with cardiovascular risk factors such as higher serum vascular cell adhesion molecule[22] as well as with prevalent and incident ischemic heart disease, stroke, and total mortality.[23] This is concordant with evidence from a cross-sectional study of Samoan Islanders that showed that lower *LINE-1* methylation was associated with higher low-density lipoprotein (LDL) and lower high-density lipoprotein (HDL) cholesterol.[24] In visceral adipose tissue samples from severely obese individuals, *LINE-1* hypomethylation was found to be associated with higher prevalence of metabolic syndrome, and therefore elevated risk for CVD.[25] Although data presented by Baccarelli et al.[23] suggest that *LINE-1*

hypomethylation precedes disease diagnosis and could be used for early identification of at-risk individuals, this suggestion has yet to be translated into clinical applications.

Candidate gene methylation studies

Complementing the global methylation studies, several reports have assessed relationships between the methylation status of *a priori*-selected, biologically relevant genomic regions and cardiovascular risk. Of particular interest among candidate gene studies are investigations of *FTO*, a prominent obesity-associated gene that has also been linked to CVD risk independently of its effect on body mass index.[45] Several studies revealed associations between sequence variation in *FTO* and levels of DNA methylation, both at the *FTO* locus[26] as well as in *KARS*, *TERF2IP*, *DEXI*, *MSI1*, *STON1* and *BCAS3*, [27] suggesting that the association between *FTO* mutations and cardiometabolic outcomes may be underpinned by epigenetics. Interestingly, the effects of *FTO* variation are mediated by regular physical activity, [46] but a recent epigenome-wide DNA methylation study in adipocytes[47] did not identify *FTO* loci as differentially affected by a six-month exercise intervention, raising the possibility of involvement for other mechanisms. Specifically, recent studies show that the FTO protein can also catalyze modifications of RNA through oxidative demethylation of N6-methyladenosine.[48,49] While the evidence from both human cells and animal tissue is compelling, the implications of these RNA modifications for disease risk warrant further study. Most recently, evidence emerged that *FTO* directly interacts at long range with the promoter of the homeobox gene *IRX3* in humans, mice, and zebrafish, enhancing *IRX3* expression; in turn, *IRX3* has a direct effect on body mass and composition, as demonstrated by knockout mouse models.[50] Such rigorous studies are poised to fill in the mechanistic gaps in our current understanding of cardiometabolic genetics and epigenetics.

Other candidate gene studies have identified a number of promising epigenetic targets in CVD. Notably, a large-scale prospective cohort study of patients with stable coronary heart disease established that methylation (in whole blood samples) of *F2RL3*, a known locus linked with tobacco use, is associated with both CVD risk factors (e.g. CRP and body mass index) at baseline as well as with overall mortality but not with CVD mortality at 8 years of follow-up.[28] Interestingly, *F2RL3* methylation status was a better prognostic factor than self-reported and cotinine-validated smoking behavior.[28] In another candidate gene study, higher leukocyte DNA methylation at *INS* and *GNASAS*, two loci previously reported to be sensitive to prenatal environment, was prospectively associated with the risk of (MI) infarction in women but not in men.[29] Cross-sectionally, hypermethylation of *PLA2G7* was associated with coronary heart disease (CHD) among females[30] and hypermethylation of a locus on 9p21, a region of robust genetic associations with CVD, was more prevalent among coronary artery disease cases in a Chinese population.[51]

Other candidate gene studies have investigated intermediate cardiovascular risk phenotypes such as obesity, dyslipidemia, or hypertension. For example, the methylation extent of *IGF2*, which encodes a key growth factor in fetal development, predicts development of obesity[31] and cross-sectionally associates with a higher triglyceride/HDL cholesterol ratio.[32] Additionally, hypermethylation of *POMC*, a gene encoding proopiomelanocortin, in

umbilical cord blood was associated with higher blood triglycerides and insulin during childhood.[33] In animal models, changes in methylation and expression of *FADS2*, a gene previously linked to cardiometabolic disease, affected arterial biosynthesis of polyunsaturated fatty acids and induced vascular dysfunction.[52,53] Another analysis of the VA Normative Aging Study related small changes in blood pressure with the methylation of genes encoding pro-inflammatory proteins such as toll-like receptor 2, inducible nitric oxide synthase, and interferon-gamma.[34] Similarly, increased methylation in the promoter region of the *HSD11B2* gene was shown to be associated with the increased activity of the 11-betaHSD2 enzyme and the risk of hypertension.[35]

Most epigenetic markers discovered through the candidate gene approach have proven to be useful in elucidating the underlying pathophysiological mechanisms, but their clinical potential, especially in comparison with traditional cardiovascular risk factors, remains unclear. Moreover, is likely that the narrow scope of candidate gene studies precludes detection of many novel methylation loci that could be germane to CVD.[54]

Epigenome-wide studies

With the introduction of the Infinium Human Methylation 450K array (Illumina, San Diego, CA) that measures DNA methylation at approximately 450,000 cytosine-phosphate-guanine (CpG) sites across the genome, the focus of cardiovascular epigenetics has shifted from candidate regions to epigenome-wide studies. A recent study from our group, conducted in CD4+ T-cells from 991 metabolically healthy European American participants of the Genetics of Lipid Lowering Drugs and Diet Network Study, has identified robust associations between the methylation status of two markers in *CPT1A*, the expression of *CPT1A*, and the levels of plasma triglycerides and very low-density lipoprotein cholesterol.[55] *CPT1A* encodes the carnitine palmitoyltransferase enzyme, which controls fatty acid flux in the liver via the beta-oxidation and esterification pathways, thus providing strong biological plausibility for the observed association. Furthermore, the direction of the association was replicated in an independent cohort.[55] Using a lower-resolution platform (Illumina Infinium Human Methylation 27), another group has identified new loci associated with HDL cholesterol in the setting of familial hypercholesterolemia.[37] Epigenome-wide studies of cardiomyopathy found distinct patterns of DNA methylation and histone-3 lysine-36 trimethylation in left ventricle tissue between cases and controls, particularly in *LY75*, *ERBB3*, *HOXB13*, *ADORA2A*, and *DUX4*; of those, *DUX4*, *LY75* and *ADORA2A* also exhibited changes in gene expression and functional relevance.[40,41] In animal models of hypertrophy, epigenome-wide studies of cardiomyocytes identified signature histone modifications and transcriptional changes, linking epigenetic processes to gene expression reprogramming implicated in heart failure.[56]

In addition to CVD phenotypes, epigenome-wide studies have shown differential methylation status of various loci associated with such CVD risk factors as age,[57] obesity,[38] air pollution,[58] measures of fasting insulin and insulin resistance,[39] and smoking,[59,60] lending support to the view of epigenetic changes as the process by which environmental factors influence heritable genetic variation. Currently several other large-scale cohorts are in process of analyzing their own genome-wide methylation data and establishing consortia

for meta-analyses, suggesting that more epigenetic biomarkers of cardiovascular risk will be uncovered in the months to come. The International Human Epigenome Consortium is in process of producing reference maps of 1,000 human epigenomes for a range of cell types, which could significantly accelerate epigenome-wide studies for many common diseases.[54] Additionally, as the results from gene expression consortia suggest, these large-scale projects may identify surrogate tissues (and/or proxy epigenetic loci) to facilitate clinical implementation of the methylation markers.

However, the findings of the epigenome-wide association studies must be interpreted in light of several important limitations. First, all of the observed associations to date have come from cross-sectional studies; thus, it is impossible to establish either temporality or causality of the relationship between cardiovascular risk markers and DNA methylation. Second, from the clinical perspective, the epigenetic biomarkers are only as good as the intermediate risk factor they represent (e.g. triglycerides) because, unlike candidate gene studies, no epigenome-wide study to date has investigated the CVD endpoints of myocardial infarction, stroke, or death from related causes. Finally, as epigenome-wide studies represent an agnostic research approach, independent replication of epigenetic findings remains the gold standard for reducing false positive results.

Crosstalk between genetic markers, DNA methylation, and gene expression

One of the factors hindering clinical implementation of the findings from candidate gene or epigenome-wide methylation studies is the complexity of the relationship between sequence variation, methylation, and gene expression patterns. The correlation between genetic polymorphisms and DNA methylation levels is well-established and predominantly accounted for by variants located at CpG sites.[61-63] Similarly, genetic variation has been shown to underlie variation in gene expression.[64] The traditional paradigm holds that DNA methylation is also associated with changes in gene expression, specifically gene repression, due to interference with the binding of transcription factors or recruitment of histone deacetylases or other repressors.[65,66] In contrast, recent studied showed that DNA methylation can affect expression positively as well as negatively, and other factors (e.g. genetic variation or environmental inputs such as drug treatment) have been postulated to affect both methylome and transcriptome.[62,65-67] It is also notable that both methylation and gene expression are dynamic processes, so their effects may be mutual and bidirectional; disentangling the causal web would require repeated measurements and considerable expenses. Because of the complexity of these interactions (Figure 1), any novel methylation markers cannot be interpreted or used outside of their genomic context, data on which are not always available. While genotype information has become commonplace with the advent of genome-wide associations studies, most population or clinical methylation studies of cardiovascular traits, with few exceptions, [37,40] do not have expression data. As a result, the mechanistic insights provided by such studies are limited, especially in light of the evidence supporting the connection between methylation and expression in recent *in vitro* studies of cardiovascular phenotypes.[68,69] However, as large-scale expression profiling techniques such as RNA-Seq become more accessible, studies will be able to better integrate data on epigenetic changes, DNA sequence variation, and gene expression, identifying the most promising targets for screening and intervention.

The aging process, which affects the methylation status of as many as 29% of CpG sites located on the Illumina 450K array in white blood cells,[70] introduces further complexity into the epigenetic paradigm. Specifically, over the course of the human lifespan the DNA methylation content decreases and patterns become less correlated between neighboring regions.[71] Although associated with normal aging processes, such changes may also underlie the development of some pathological conditions, including CVD. Finally, the consequences of aging may be mediated by trans-generational effects, with methylation at highly heritable regions shown to be more stable over time and functionally relevant.[72] Trans-generational effects also occur with other CVD risk factors, most notably smoking and other environmental chemicals,[73] via in utero exposure or the paternal germline.[74] As a result, the influences of inherited (as in the case of transgenerational effects) or environmental factors on CVD risk are difficult to distinguish from the cardiovascular implications of epigenetic processes themselves.

Epigenetic markers in CVD prevention and treatment

Because DNA methylation is intrinsically reversible, it presents a lucrative target for interventions in cardiovascular disease. These interventions can be either lifestyle-related or pharmacological. In the lifestyle domain, gene-diet interactions are often underpinned by epigenetic mechanisms, giving rise to the idea of nutrition as "epigenetic medicine."[75,76] The nutriepigenetic paradigm was first proposed in the context of atherosclerosis, suggesting that global hypomethylation due to reduced folic acid and vitamin B12 levels increases cardiovascular risk;[77] however, subsequent studies have failed to convincingly support this hypothesis.[78] An emerging body of data points to the role of other dietary factors (e.g. polyphenols, catechins, bioflavonoids, or selenium) in the methylation process.[79-81] For example, a recent study showed that consuming cocoa, a rich source of polyphenols, decreases the global DNA methylation of peripheral leukocytes in humans with cardiovascular risk factors.[82] While an intriguing possibility, the epigenetic link between consumption of certain nutrients and cardiovascular risk remains to be directly tested. Most likely, because of their relatively low concentrations in common foods and low bioavailability, the effect of any given compound on CVD risk is negligible.[79] Despite the lack of supporting evidence, many functional foods or supplements that market themselves as nutriepigenetic modulators are currently under development or available to consumers.[76]

Studies of associations between DNA methylation patterns and phenotypic responses to diet and/or dietary changes represent another nutriepigenetic facet of cardiovascular risk. Two trials from the same research group have identified that differential methylation of seven genes (*ATP10A*, *CD44*, *AQP9*, *DUSP22*, *HIPK3*, *TNNI3*, and *TNNT1*) as prognostic biomarkers of response to a weight loss intervention, and also reported diet-induced changes in methylation of *WT1* and *ATP10A* at the end of the study.[83,84] Because these studies were conducted in adolescents, the effect of the same markers on CVD endpoints was not investigated. In a small study of obese adults, hypermethylation of *SERPINE1*, a gene encoding plasminogen activator inhibitor 1 that had previously been associated with metabolic dysfunction, was prospectively associated with not only weight loss but also more drastic reductions in total cholesterol and triglycerides.[85] These findings provide important etiological clues to the pathogenesis of chronic disease, but their clinical implications are not

straightforward. First, some of the strongest predictors of both DNA methylation and dietary response are non-modifiable factors like age and sex,[86,87] which could confound the observed associations and limit the prognostic value of the biomarkers. Second, none of the above-referenced studies measured gene expression, further limiting insights into the underlying mechanisms. Third, larger sample sizes and longer intervention periods would enhance precision and clinical relevance of the findings.

In contrast to the uncertain nature of nutriepigenomic findings, the cardiovascular implications of pharmacoepigenetics appear to be more direct and far-reaching. A growing body of evidence shows that epigenetic modifications could be reversible through both novel pharmaceutical approaches such as bromodomain and extra-terminal (BET) protein inhibitors as well as commonly used drugs.[88,89] Some examples of common drugs with newly discovered epigenetic effects are vasodilators such as hydralazine, which has been shown to inhibit DNA methylation by either interfering with DNA methyltransferase directly or by reducing its gene expression, and procainamide, a sodium channel blocker that inhibits DNA methyltransferase I.[90-92] A serious side effect common to both hydralazine and procainamide is an autoimmune disease similar to lupus, believed to be triggered by extensive hypomethylation across the genome.[90,93] Both of these therapeutic approaches are now considered a promising avenue for treating cancer,[94] but the cardiovascular implications of their epigenetic effects remain unclear.

The evidence in support of epigenetic effects of statins paints a complex picture. One group of studies demonstrated that trichostatin A can inhibit histone deacetylases (HDACs) in cancer cells and mouse models, reducing deacetylation and thus increasing transcription.[7,95,96] Initially described as repressors of transcription because of their effects on histones, HDACs have since been shown to act on other cellular substrates and activate transcription as well, e.g. by deacetylating a key member of the mitogen-activated kinase (MAP) family.[97-99] Because the cardioprotective potential of such gene upregulation has not been demonstrated and animal studies documented adverse morphological effects instead, drug development to date has focused on downregulating HDACs.[98] A number of preclinical trials have highlighted the potential of HDAC inhibitors, particularly in the context of heart failure and myocardial injury.[95] In animal and cell culture models, HDAC inhibitors such as trichostatin A have been shown to reduce inflammation and block adverse cardiac remodeling.[100] While the underlying mechanism remains largely unknown, recent studies implicated signaling molecules MKK3 and Akt-1 as well as gp-91, a subunit of HADPH-oxidase, in HDAC-inhibition-induced cardioprotection.[101,102] The anti-inflammatory effect of HDAC inhibitors may be due to induction of regulatory T-cells shown with statin therapy in patients with acute coronary syndrome.[100,103] Furthermore, HDAC inhibitors may be beneficial in treatment of cardiac fibrosis associated with activation and proliferation of fibroblasts.[104] However, further mechanistic questions must be answered for effective design of pharmaceutical interventions, e.g. which of the over a dozen HDAC genes and their many mRNA isoforms are particularly salient in the setting of CVD?[100,105] As most clinical data on HDAC inhibitors come from cancer trials, the requisite dose for effective treatment of heart failure with minimal side effects remains to be determined as well, although studies of HDAC inhibition in autoimmune disease suggest that it may be lower than in the cancer setting.[100,106]

In contrast to the findings of increased gene expression as a result of HDAC inhibition, a study of cultured human endothelial cells[107] showed that statin-induced histone modification can also reduce gene expression, accounting for some of the anti-inflammatory benefits of statin therapy. However, while promising in the context of cancer treatment,[108] the epigenetic effects of statins in the setting of CVD are likely to be pleiotropic, leading to both positive and negative consequences. For example, one such effect was shown to be decreased expression of the atrogin-1 gene, which causes muscle fiber damage, a common side effect of statin therapy.[88109110] It is evident that any therapeutic approaches that aim to harness the epigenetic potential of statins and other HDAC inhibitors will need to address this problem of unspecific effects, requiring many more studies of safety and efficacy.

At the cutting edge of epigenetic therapies for CVD is RVX-208, a small molecule BET bromodomain inhibitor that targets HDL cholesterol levels by inducing apolipoprotein A-I (apoA-I).[111] In addition to its effects on apoA-I, BET inhibition suppresses cardiomyocyte hypertrophy *in vitro* and pathological cardiac remodeling *in vivo* by mediating transcriptional pause release in heart failure, suggesting pleiotropic effects on cardiovascular health.[112] Two clinical trials have now been completed evaluating the safety and efficacy of RVX-208 in patients with CVD who were simultaneously receiving statin therapy, and the results were conflicting. The Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I stimulation (SUSTAIN) assessed safety as well as changes in HDL cholesterol and apoA-I levels and found increases in both parameters compared with placebo over 24 weeks.[113,114] There was no evidence of additional hepatotoxicity in that study population.[114] On the other hand, the results of the ApoA1 Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation (ASSURE) trial, which used intravascular ultrasonography to assess changes in atheroma volume after 26 weeks of RVX-208 therapy, failed to detect statistically significant differences between the treatment and placebo group.[115] Similarly, the investigators of ASSURE did not observe the beneficial changes in apoA-I or HDL-cholesterol that were reported in the SUSTAIN study.[115] Most recently, when SUSTAIN and ASSURE data on major adverse cardiovascular events (CVD deaths, nonfatal myocardial infarctions, angina pectoris, or revascularizations for progressive coronary artery disease) were analyzed jointly, the event rate was considerably lower (6% vs. 21%, P=0.007) in the RVX-208 group compared with placebo.[116] While these findings are undoubtedly promising, they are far from conclusive and require independent replication. Additionally, it is unclear whether the beneficial effects of RVX-208 would translate to the setting of primary prevention.

In summary, the development of clinical applications of cardiovascular epigenetics is still in its infancy. While candidate gene and epigenome-wide studies of DNA methylation have yielded promising findings (Table 1) that have elucidated underlying biological pathways, these markers have yet to be translated into meaningful prognostic markers or intervention targets, mostly because of their temporal instability and the complexity of the relationships between genetic variation, gene expression, and epigenetic processes. Therapeutic approaches that target epigenetic processes in the cardiovascular system are emerging, but clinical evidence of their safety and effectiveness in humans is extremely limited. Several research directions represent particularly promising avenues of investigation: 1) testing the

prognostic ability of epigenetic tags against or as a supplement to traditional CVD risk stratification tools, e.g. the Framingham Risk Score; 2) development of more sensitive and specific pathophysiologic markers of CVD, e.g. microRNAs as diagnostic tools for acute MI,[117,118] and 3) development of therapeutic and preventative approaches specifically targeting epigenetic processes in the cardiovascular system. On balance, the clinical potential of cardiovascular epigenetics still has a long road to fulfillment.

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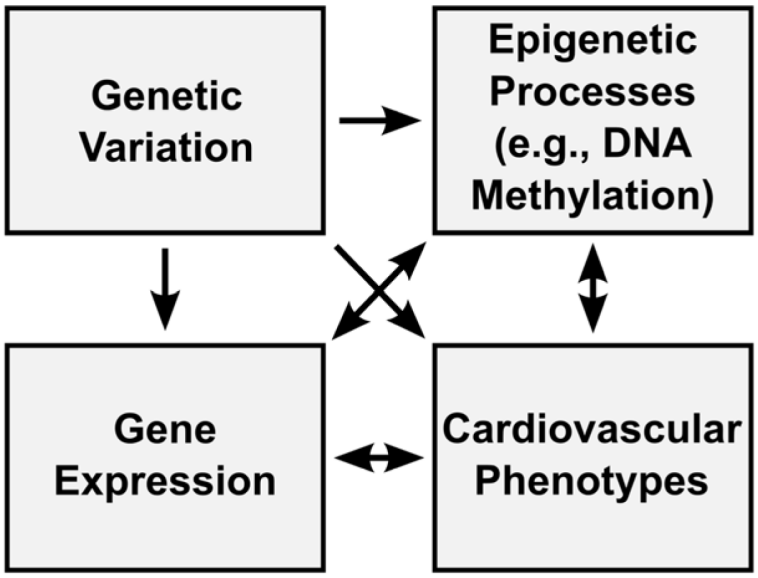

Figure 1.

An integrated perspective on genomic variation, gene expression, and epigenetic modification in cardiovascular phenotypes. While DNA sequence variation necessarily precedes changes in both gene expression and epigenetic processes, all other relationships are bidirectional.

Table 1

Epigenetic markers and their associated cardiovascular disease or phenotype.

Epigenetic marker	Associated condition/phenotype	Ref
Global methylation studies		
increased homocysteine, ↓ GM	↑ vascular disease, coronary heart disease	18,19
↑ GM	↑ systemic inflammation, mortality from chronic kidney disease	20
↑ Alu	environmental pollution impact on inflammatory markers	21
↓ LINE-1	↑ inflammatory marker, ischemic heart disease, stroke, total mortality, LDL, metabolic syndrome, ↓ HDL	22-25
Candidate gene studies		
↑ *FTO*	↑ obesity	26
↓ CERCAM, ↓ DPYD, ↑ IL12A, ↓ ZNF35, ↓ ZNF362, ↑↓ Others	↑ obesity	27
↑ *F2RL3*	↑ CVD risk factors, total mortality	28
↑ *INS, GNASAS*	↑ MI risk in females	29
↑ *PLA2G7*	↑ CHD in females	30
↑ *IGF2*	↑ obesity development, TG/HDL ratio	31,32
↑ *POMC*	↑ childhood TG, insulin	33
↑ *TLR2, NOS2*	↑ blood pressure	34
↑ *IFNG*	↓ blood pressure	34
↑ *HSD11B2* promoter	↑ hypertension risk	35
Epigenome-wide studies		
↑ *CPT1A*	↑ TG, VLDL	36
↑ *TNNT1* promoter	↑ HDL particle size, HDL-C	37
↑ at multiple loci	↑ obesity	38
↑ *ABCG1*	↑ fasting insulin	39
↑ LY75, ↓ ERBB3, ↓ HOXB13, ↓ ADORA2A, ↑ DUX4	↑ cardiomyopathy	40,41

↑, increased methylation at locus or increase in phenotype metric; ↓, decreased methylation at locus or decrease in phenotype metric; CHD, coronary heart disease; CVD, cardiovascular disease; GM, global methylation; HDL-C, high-density lipoprotein cholesterol; HDL, highdensity lipoprotein; LDL, low-density lipoprotein; LINE-1, long interspersed nucleotide element-1; MI, myocardial infarction; Ref, reference; TG, triglycerides; VLDL, very low-density lipoprotein