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REVIEW ARTICLE

Epigenetic regulation of cardiovascular diseases induced by behavioral and environmental risk factors: Mechanistic, diagnostic, and therapeutic insights

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Abstract

Behavioral and environmental risk factors are critical in the development and progression of cardiovascular disease (CVD). Understanding the molecular mechanisms underlying these risk factors will offer valuable insights for targeted preventive and therapeutic strategies. Epigenetic modifications, including DNA methylation, histone modifications, chromatin remodeling, noncoding RNA (ncRNA) expression, and epitranscriptomic modifications, have emerged as key mediators connecting behavioral and environmental risk factors to CVD risk and progression. These epigenetic alterations can profoundly impact on cardiovascular health and susceptibility to CVD by influencing cellular processes, development, and disease risk over an individual's lifetime and potentially across generations. This review examines how behavioral and environmental risk factors affect CVD risk and health outcomes through epigenetic regulation. We review the epigenetic effects of major behavioral risk factors (such as smoking,

Abbreviations: 3D, three-dimensional; 5aza2DC, 5-aza-2′-deoxycytidine; 5mC, 5-methylcytosine; AHR, aryl hydrocarbon receptor; AHRR, aryl hydrocarbon receptor repressor; AID/APOBEC, activation-induced cytidine deaminase/apolipoprotein B mRNA-editing enzyme complex; ASO, antisense oligonucleotide; ATAC-seq, assay for transposase-accessible chromatin sequencing; BC, black carbon; CHD, chromodomain helicase DNA-binding; cirRNAs, circular RNAs; COPD, chronic obstructive pulmonary disease; CRISPR-Cas, clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins; CVD, cardiovascular disease; DNase-seq, DNase I hypersensitive site sequencing; DNMTs, DNA methyltransferases; DOCA, deoxycorticosterone acetate; EHMT2, euchromatic histone lysine methyltransferase 2; EVs, extracellular vesicles; F2RL3, factor II receptor-like 3; FISH, fluorescence in situ hybridization; H3/H4, histone 3/4; HAT, histone acetyltransferases; HDACs, histone deacetylases; IL-1β, interleukin-1β; INO80, inositol requiring 80; iPSCs, induced pluripotent stem cells.; ISWI, imitation switch; LINE1, long interspersed nuclear element-1; lncRNAs, long noncoding RNAs; MBD, methyl-CPG-binding domain; Me, methylation; Me1, mono-methyl; Me2, di-methyl; Me3, tri-methyl; MedDiet, Mediterranean diet; medip-seq, methylated DNA immunoprecipitation sequencing; MI, myocardial infarction; miRNAs, microRNAs; mtROS, mitochondrial reactive oxygen species; NAD+, nicotinamide adenine dinucleotide; ncRNAs, non-coding RNAs; NGS, next-generation sequencing; NO, nitric oxide; PAR4, protease-activated receptor-4; PCR, polymerase chain reaction; PHGDH, phosphoglycerate dehydrogenase; piRNAs, PIWI-interacting RNAs; RRBS, reduced representation bisulfite sequencing; SAM, S-adenosylmethionine; scATAC-seq, single-cell sequencing assay for transposase-accessible chromatin; scHiC, single-cell Hi-C; scRRBS, single-cell RRBS; siRNAs, short interfering RNAs; SIRT1, sirtuin 1; SKOR2, SKI family transcriptional corepressor 2; SLC7A11, solute carrier family 7 member 11; SMUG1, single-strand-selective monofunctional uracil-DNA glycosylase 1; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; SO4, sulfates; STAR, steroidogenic acute regulatory protein; SULF1, sulfate endosulfatase; SWI/SNF, switching defective/sucrose nonfermenting; TDG, thymine DNA glycosylase; TET, ten-eleven translocation; TNFα, tumor necrosis factor-α; TSPAN2, tetraspanin 2; UHRF, ubiquitin-like containing PHD and RING finger domain; VCAM-1, vascular cell adhesion molecule 1; wgbs-seq, whole-genome bisulfite sequencing.

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alcohol consumption, physical inactivity, unhealthy diet, and obesity) and environmental risk factors (including air and noise pollution) in the context of CVD pathogenesis. Additionally, we explore epigenetic biomarkers, considering their role as causal or surrogate indicators, and discuss epigenetic therapeutics targeting the mechanisms through which these risk factors contribute to CVD. We also address future research directions and challenges in leveraging epigenetic insights to reduce the burden of CVD related to behavioral and environmental factors and improve public health outcomes. This review aims to provide a comprehensive understanding of behavioral and environmental epigenetics in CVD and offer valuable strategies for therapeutic intervention.

KEYWORDS

behavioral and environmental factors, biomarkers, cardiovascular disease, epigenetics, therapeutics

1 | INTRODUCTION

According to the World Health Organization (WHO), cardiovascular disease (CVD) is the leading cause of death globally, accounting for an estimated 17.9 million deaths annually, or approximately 31% of all global deaths.[1] Notably, a substantial proportion (80%) of CVD cases are preventable or treatable through interventions targeting environmental factors, such as air and noise pollution, and behavioral risk factors, including tobacco use, alcohol consumption, physical inactivity, unhealthy diet, and obesity.[2] It is important to note that reducing behavioral and environmental risk factors through lifestyle adjustment such as adopting a healthy diet, regular exercise, smoking cessation, and maintaining a healthy weight can significantly reduce the risk of developing CVD and improve overall cardiovascular health.[3] On the other hand, understanding the mechanisms underlying behavioral and environmental factors-induced CVD holds great promise for the development of innovative therapies for CVD, offering new avenues for disease prevention, management, and personalized treatment strategies.[4]

Epigenetics is a regulatory mechanism that can alter gene expression, function, and activity without modifying the original DNA sequence.[5] It is considered as the major regulation mechanism for the cell response to behavioral and environmental risk factors-related CVD.[6,7] First, large-scale twin studies have demonstrated that despite sharing identical genetics, monozygotic twins often exhibit discordance in CVD risk factors and outcomes.[8–10] The degree of epigenetic differences between twins was found to correlate with the amount of time spent apart and differences in their CVD histories.[8–10] This indicates that substantial behavioral and environmental influences, such as diet and lifestyle, along with epigenetic mechanisms, can account for these discrepancies. Second, epigenetics serves as a key mechanism controlling cell and tissue differentiation by partitioning the genome into transcriptionally active and quiescent domains during cardiovascular system development.[11–13] A great variety of external environmental factors can alter epigenetic programs in multiple cells and tissues and thereby heighten or lessen the risk of CVD.[14,15] Third, epigenetic alterations are one of the key features for age-related CVD.[16–19] Epigenetic dysregulation comprising local and global changes in DNA and histone modifications, transcription factor binding, disorganization of the nuclear lamina, RNA modifications, and misfolding of the genome are hallmarks of aging-related CVD, leading to senescent cell burden, proinflammatory sequelae, cardiomyocyte hypertrophic growth, vascular remodeling, calcification, and fibrosis.[16,20–22] Collectively, these findings support the concept that epigenetic modifications are subject to behavioral and environmental exposure throughout our lives, which affects CVD susceptibility and ultimately health outcomes.

It is important to understand that epigenetic modifications are different from genetic changes. Unlike changes to the DNA sequence, which are generally permanent, epigenetic modifications can be reversible.[23] This dynamic nature allows cells to adjust their gene expression patterns in response to behavioral and environmental factors, enabling flexibility in cellular function. Epigenetic modifications can be influenced by different types of risk factors such as diet, stress, toxins, and lifestyle choices.[24] This behavioral and environmental plasticity allows for adaptive responses to changes in the external environment and can contribute to CVD variability within populations. However, some epigenetic modifications are highly stable and can be passed

from parent cells to daughter cells during cell division, allowing for the maintenance of cell identity and function across generations of cells.[25] In some cases, epigenetic changes can also be inherited across generations of organisms, influencing traits and phenotypes without altering the DNA sequence.[26] For example, recent studies have demonstrated that epigenetic changes, such as those induced by environmental factors or toxicants during critical periods of development, can be inherited across generations of organisms, leading to transgenerational diseases[27,28] These features of epigenetic modifications are crucial for biomarker discovery and drug development for CVD patients.

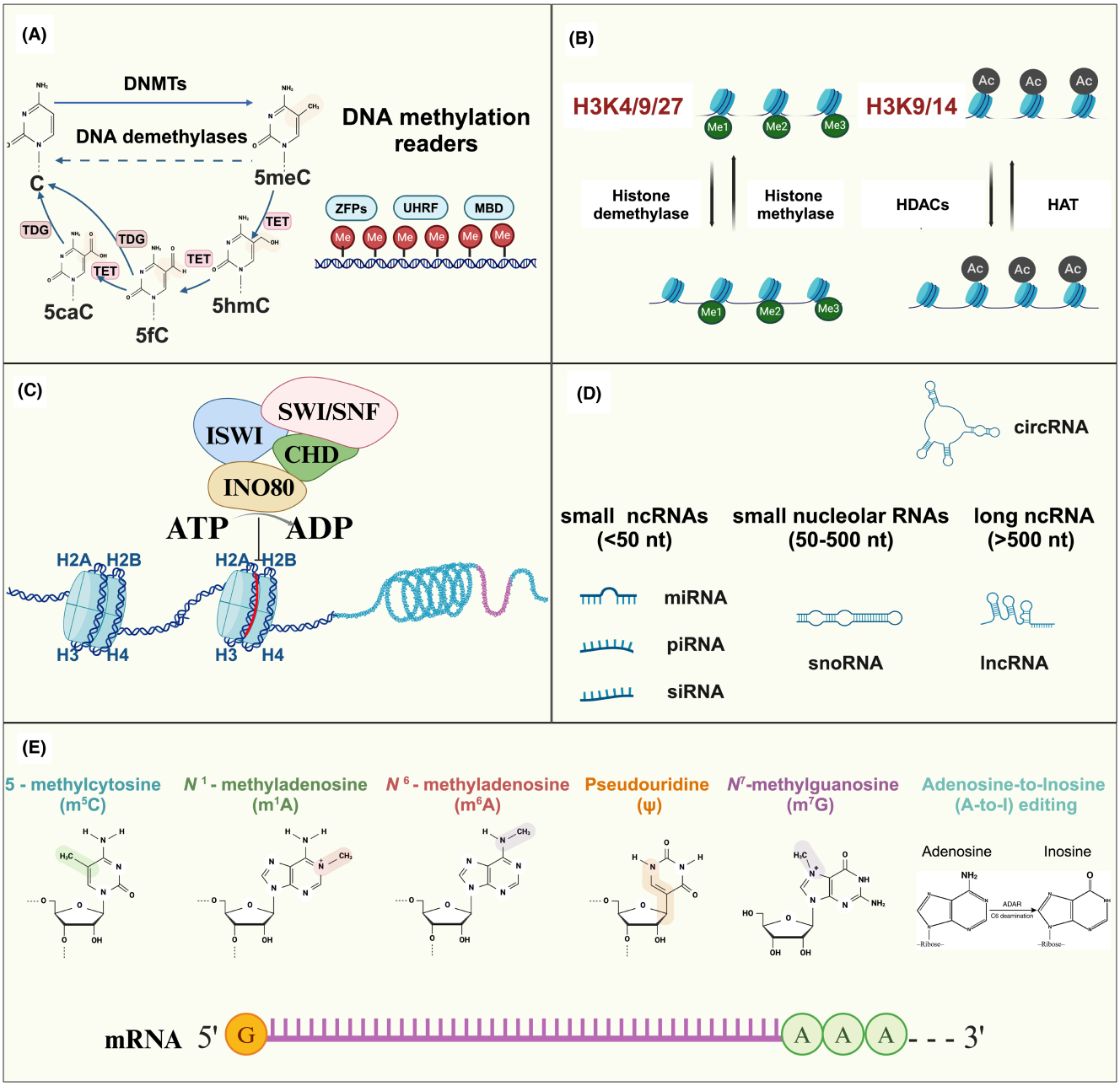
In this review, we will consolidate recent research on how behavioral and environmental factors modulate epigenetic patterns and induce CVD, encompassing DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs (ncRNAs).[23] Our primary focus will be on modifiable risk factors such as smoking, alcohol consumption, diet, physical inactivity, obesity, and air pollution.[29] Additionally, we will explore the potential applications of epigenetics in developing innovative biomarkers and therapeutics for patients with CVD.

2 | EPIGENETIC REGULATORY MECHANISMS

Chromatin is a complex of DNA and histone proteins that make up the chromosomes within the nucleus of eukaryotic cells. The basic repeating unit of chromatin is the nucleosome, which consists of DNA wrapped around a core of histone proteins.[30] Chromatin can exist in different states of compaction, ranging from condensed and transcriptionally inactive (heterochromatin) to more open and transcriptionally active (euchromatin). Changes in chromatin structure can influence the accessibility of DNA to transcriptional machinery and thus regulate gene expression.[31] Epigenetic modifications can affect chromatin structure by altering the interactions between DNA and histones or changing RNA function by chemical modifications on RNA, thereby influencing gene transcription activities. Epigenetic regulation is mediated by modifications to DNA (such as DNA methylation), RNA (such as epitranscriptomic modification), and histones (such as histone acetylation, methylation, and phosphorylation), chromatin remodeling (such as nucleosome positioning and interactions between nucleosomes and other regulatory proteins regulation), as well as by the actions of ncRNAs. Aberrant epigenetic modifications can lead to altered gene expression patterns and contribute to various human diseases, including cancer, neurological disorders, autoimmune diseases, and CVD.[32]

2.1 | DNA methylation

DNA methylation is a covalent modification involving the addition of a methyl group to the cytosine (C) 5 position of cytosine residues. While predominantly occurring within cytosine-phosphate-guanine (CpG) dinucleotide contexts, non-CpG methylation, including cytosine-phosphate-adenine (CpA), cytosine-phosphate-thymine (CpT), and cytosine-phosphate-cytosine (CpC) motifs, has been identified, particularly in embryonic stem cells and specific neuronal cell types.[33,34] It stands as one of the most extensively studied epigenetic mechanisms, exerting significant influence over genome stability, transcriptional regulation, and developmental processes.[35] DNA methylation suppresses gene transcription directly, by impeding the binding of transcription factors to DNA or, indirectly, by recognition of methylated sites by chromatin-modifying enzymes.[36] The dynamic equilibrium of genomic methylation is meticulously maintained by DNA methyltransferases (DNMTs) and demethylases (Figure 1A). Enzymes involved in DNA methylation can be divided into three categories according to their roles in DNA methylation: writing enzymes, erasing enzymes, and reading enzymes. Writing enzymes, including DNMT1, DNMT3a, and DNBT3b,[37] catalyze the addition of methyl groups to cytosine residues. DNMT1 can not only accurately replicate the original methylation pattern before DNA replication, but also repair DNA methylation during DNA replication. DNMT3a and DNMT3b, also referred to as de novo DNMTs, possess the ability to introduce new methylation into naked DNA, thus establishing novel methylation patterns for previously unmodified DNA. DNMT3a and DNMT3b target specific DNA sequences and perform de novo DNA methylation through binding to transcription factors or repressor complexes.[38] Eraser enzymes play a crucial role in modifying and removing DNA methyl groups. For example, activation-induced cytidine deaminase/apolipoprotein B mRNA-editing enzyme complex (AID/APOBEC),[39] the human ten-eleven translocation (TET) family members,[40] TET1, TET2, and TET3, thymine DNA glycosylase (TDG), and single-strand selective monofunctional uracil-DNA glycosylase 1 (SMUG1)[41] are key catalytic enzymes facilitating the demethylation process of 5mC DNA. Reading enzymes can recognize and bind methyl groups to affect gene expression. Proteins belong to the reading enzymes (adaptors) are mainly involved in regulating gene expression. Three protein families recognize DNA methylation: the methyl-CpG-binding domain (MBD) proteins, the ubiquitin-like containing PHD and RING finger domain (UHRF) proteins, and specific zinc finger proteins (Figure 1A).[42] The zinc finger proteins include krüppel-like factor 4 (KLF4), zinc finger and BTB domain containing 33 (ZBTB33), zinc finger protein 57



homolog (ZFP57), Wilms' tumor protein 1 (WT1), early growth response 1 (EGR1), and CCCTC-binding factor (CTCF).[43] Aberrations in genomic methylation homeostasis contribute to various diseases, including CVD.[44] Environmental factors can affect DNA methylation either by directly or indirectly suppressing DNA methylation via changing the availability of substrates required for the enzymatic reactions.[45,46] For instance, Reichard et al. observed that arsenite reduces the expression of the DNA methyltransferase genes DNMT1 and DNMT3A in human HaCaT keratinocytes.[47] The proposed mechanism suggests that arsenic regulates DNMT expression by interfering with the retinoblastoma protein (RB)/E2F transcription factor (E2F) transcriptional axis.[48]

2.2 | Histone modification

The modification of histone is an important posttranslational process that includes acetylation, methylation, phosphorylation, and ubiquitination. These processes play key roles in gene regulation by influencing the affinity between transcription factors and gene promoters.[49] Histone acetylation and methylation are the two most well-studied histone modifications. Histone acetylation mainly occurs at the more conserved lysine sites at the N-terminus of H3 and H4. It is coordinated by histone acetyltransferases (HAT) and histone deacetylases (HDACs) (Figure 1B).[50] Histone methylation is a posttranslational modification that involves the addition of methyl groups to histone

FIGURE 1 Types of epigenetic modifications. (A) DNA methylation: DNA methylation predominantly occurs at cytosine-phosphate-guanine (CpG) sites but can also be found at non-CpG sites. Unlike promoting gene transcription, CpG methylation within gene promoter regions is typically associated with transcriptional repression and decreased gene expression. The process of DNA methylation involves "writing" enzymes (DNA methyltransferases, DNMTs), "erasing" enzymes (DNA demethylases, including TET: ten-eleven translocation proteins), and "reading" enzymes (methyl-CpG-binding domain proteins, MBD; ubiquitin-like containing PHD and RING finger domain proteins, UHRF; and zinc finger proteins, ZFPs). TDG (thymine DNA glycosylase), 5hmC (5-hydroxymethylcytosine), 5fC (5-formylcytosine), 5meC (5-methylcytosine), 5caC (5-carboxylcytosine). (B) Histone modifications: histone methylation and acetylation are two of the most extensively studied histone modifications. Histone methylation is mediated by histone methyltransferases and opposed by histone demethylases. Histone acetylation is facilitated by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs). Key histone marks include H3K9 (histone H3 lysine 9), H3K4 (histone H3 lysine 4), H3K14 (histone H3 lysine 14), and H3K27 (histone H3 lysine 27). Me (methylation), Me1 (mono-methylation), Me2 (di-methylation), Me3 (tri-methylation), Ac (acetylation), H2A/H2B/H3/H4 (histone H2A/H2B/H3/H4). (C) Chromatin remodeling: chromatin remodelers facilitate chromatin assembly and reorganization by repositioning or evicting histone octamers, thus allowing nucleosome sliding, eviction, or localized unwrapping. This remodeling also includes altering nucleosome composition through the exchange of histone dimers. Major chromatin remodeler complexes include SWI/SNF (switching defective/sucrose nonfermenting), ISWI (imitation switch), CHD (chromodomain helicase DNA-binding), INO80 (inositol requiring 80), and DBP (DNA-binding protein). (D) Noncoding RNAs: Overview of different categories of noncoding RNAs, classified by their size and function, and their roles in epigenetic regulation. (E) Epitranscriptomic modifications: Various RNA modifications that contribute to the regulation of gene expression at the posttranscriptional level. These include m6A (N6-adenosine methylation), m5C (5-methylcytosine), Ψ (pseudouridine), m7G (N7-methylguanosine), m1A (N1-adenosine methylation), and A-to-I editing (adenosine-to-inosine).

proteins, particularly on lysine or arginine residues. While histone acetylation usually increases gene transcriptional activity through adding a single acetyl group to each amino acid residue; histone methylation (Me), found as mono-methyl (Me1), di-methyl (Me2), and tri-methyl (Me3) group states can inhibit or increase gene expression depending on the amino acid position being modified.[51] DNA methylation and histone lysine methylation are intricately interconnected.[52] For example, genome-wide analyses have revealed a strong correlation between DNA methylation and histone modifications, particularly H3K4me2 and H3K4me3, suggesting a synergistic role in regulating chromatin structure and gene expression.[53] Histone phosphorylation and ubiquitination are involved in a range of cellular processes, such as DNA damage responses, transcriptional regulation, and chromatin remodeling.[54–56] For instance, phosphorylation of serine 10 on histone H3 (H3S10ph) is a histone modification, playing dual roles in seemingly opposite processes: facilitating transcriptional activation and chromatin relaxation, or chromosome compaction during cell division,[57] while H2AX phosphorylation at Serine 139 (γH2AX) plays a crucial role in the DNA damage response.[58] Moreover, histone ubiquitination, such as H2A at Lysine 119 (H2AK119ub) and H2B at Lysine 120 (H2BK120ub), is vital for gene silencing and transcriptional activation,[59] respectively, underscoring the intricate regulatory mechanisms of these modifications.

In parallel to DNA methylation, environmental factors exert profound effects on histone modifications, by directly inhibiting enzymes involved in these processes or by altering the availability of substrates essential for enzymatic reactions.[60] Acetylation and methylation of lysine residues in the amino termini of histone 3 and 4 (H3 and H4, respectively) are among the most well-studied histone modifications that could be potentially regulated by behavioral and environmental factors. Abnormal histone modification can result in an imbalance in the expression of genes associated with CVD, resulting in changes in cellular phenotypes and cardiac function.[61] Taken together, histone modifications represent a complex and dynamic system regulating gene expression under the influence of behavioral and environmental factors.

2.3 | Chromatin remodeling

Chromatin remodeling refers to the dynamic changes in the structure and organization of the chromatin, the complex of DNA and proteins that make up chromosomes within the nucleus of eukaryotic cells. These changes occur in response to various cellular signals and play essential roles in regulating DNA replication, repair, gene expression, and other nuclear processes.[62] The basic structural unit of chromatin is the nucleosome, which consists of approximately 146 base pairs of DNA surrounding a histone octamer core that contains two molecules of the core histones H2A, H2B, H3, and H4. Chromatin remodeling involves the alteration of nucleosome positioning, histone modifications, as well as interactions between nucleosomes and other regulatory proteins. These changes can result in the relaxation or condensation of chromatin, thereby influencing the accessibility of DNA to transcription factors and other regulatory proteins. There are four

major families of SWI-like ATP-dependent chromatin remodeling complexes: SWI/SNF (switching defective/sucrose nonfermenting), ISWI (imitation switch), CHD (chromodomain helicase DNA-binding), and INO80 (inositol requiring 80) complexes (Figure 1C).[63] All members of the families share an evolutionarily conserved SWI-like ATPase catalytic domain, but each one has its own distinct flanking domains. These families represent some of the major classes of SWI-like ATP-dependent chromatin remodeling complexes, each with distinct functions and regulatory roles in chromatin dynamics and gene expression. Alterations of the subunits of SWI-like ATP-dependent chromatin remodeling complexes by environmental factors (e.g., inactivating mutations, homozygous deletions, silencing, and overexpression) have been demonstrated to associate with CVD occurrence and development.[64]

2.4 | Noncoding RNAs

About 98% of the human genome does not encode proteins but can engage in transcription producing numerous ncRNAs that are transcribed from DNA without translating into proteins. They are functional RNA molecules that play important regulatory roles in various cellular processes.[65] ncRNAs can be classified into several categories based on their size and function (Figure 1D). (1) <50 nt—small noncoding RNAs: these include microRNAs (miRNAs), short interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs). They are typically around 20–30 nucleotides in length and are involved in posttranscriptional gene regulation by binding to complementary sequences in target mRNAs, leading to their degradation or translational repression. Small ncRNAs play key roles in gene expression, development, and disease.[66] (2) 50–500 nt—small nucleolar RNA (snoRNA): snoRNA biological function was initially found to modify rRNA. Small nuclear RNA (snRNA): snRNA interacts with protein factors to form small nuclear ribonucleoprotein particle and performs the function of splicing mRNA.[67] (3) >500 nt—long noncoding RNAs (lncRNAs): lncRNAs are involved in diverse regulatory processes, including chromatin remodeling, transcriptional regulation, and posttranscriptional gene regulation including mRNA splicing. They can act as scaffolds for protein complexes, guides for chromatin-modifying enzymes, or decoys for RNA-binding proteins, thereby influencing gene expression and cellular function.[68] (4) Circular RNAs (cirRNAs): circRNA molecules are covalently closed circular RNA molecules formed by back-splicing of linear pre-mRNA transcripts. circRNAs are abundantly expressed in eukaryotic cells and can act as miRNA sponges, regulate alternative splicing, or interact with RNA-binding proteins, thereby modulating gene expression and cellular processes.[69] ncRNAs expression is cell- and organ-specific. So far, many ncRNAs have been found to be associated with the physiological and pathophysiological processes of CVD in response to environmental risk factor exposure, including coronary heart disease, myocardial infarction, and vascular calcification.[70] ncRNAs related to CVD exist in human blood, urine, and other body fluids, suggesting ncRNAs hold promise as non-invasive biomarkers for disease diagnosis, prognosis, and therapeutic response prediction.[71]

2.5 | Epitranscriptomic modification

Epitranscriptomic modification refers to chemical modifications on RNA molecules. These modifications primarily include N6-methyladenosine (m^6A), 5-methylcytosine (m^5C), pseudouridine (Ψ), N7-methylguanosine (m^7G), N1-methyladenosine (m^1A), and adenosine-to-inosine (A-to-I) editing.[72] These modifications can influence a wide range of RNA functions, including transcription, translation, splicing, and degradation.[73] To date, more than 100 chemical modifications of RNA have been identified.[74] Among various modifications, m^6A modification is recognized as the most abundant and is widely distributed in both mRNA and ncRNAs.[75,76] Similar to DNA methylation and histone modification, epitranscriptomic modifications are also regulated by enzymes known as writers, readers and erasers.[77,78] Alterations or dysregulation of epitranscriptomic modification enzymes have been strongly associated with CVD.[79] For example, the m^6A RNA methylase, methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit (METTL3), can promote cardiomyocyte hypertrophy, and cardiac-specific knockout METTL3 in mice leads to morphological and functional signs of heart failure with aging and stress.[80] The m^6A modification reader protein, YTH N6-methyladenosine RNA binding protein 2 (YTHDF2), has also been shown to control cardiac function, as deletion of YTHDF2 in cardiomyocytes results in cardiac hypertrophy and increased fibrosis during cardiac injury and aging.[81] Studies suggested m^6A methylation on lncRNA mainly affects their stability.[82–84] One study showed m^6A methylation of lncRNA growth arrest-specific 5 (GAS5) by METTL3 promotes its degradation, leading to changes in cardiac fibroblast proliferation and migration in a YTHDF2-dependent manner.[83] Another lncRNA, TINCR ubiquitin domain containing (TINCR), is regulated by METTL14-dependent m^6A methylation and subsequently subjected to YTHDF2-dependent degradation. TINCER further regulates pyroptosis and diabetic cardiomyopathy through interacting with pyroptosis-related protein NLR family pyrin domain containing 3 (NLRP3).[84] In addition

to the lncRNA, emerging studies have demonstrated that modifications also occur in small ncRNAs, including miRNAs and piRNAs.[85] For example, miR-133a has been shown to be repressed upon m^{6}A modification during cardiac development and hypertrophy.[86] Furthermore, METTL3 has been identified as a positive regulator of the maturation process of pri-miR-221/222 in an m^{6}A-dependent manner, playing a critical role in angiotensin II-induced cardiac hypertrophy.[87]

3 | EPIGENETIC TECHNOLOGIES

Precisely and quantitatively mapping different known types of epigenetic modifications in histone, DNA, and RNA is essential for unraveling their roles in gene regulation in diverse biological processes. Initial methods of identifying these epigenetic modifications relied on measurements such as immunofluorescence and immunoprecipitation. These methods, however, could not provide sequence-specific information about these modifications.[88] Later, semiquantitative methods for epigenetic modifications were developed utilizing affinity enrichment (i.e., immunoprecipitation and biotin pull-down) and polymerase chain reaction (PCR) amplification to determine the epigenetic status of the gene of interest.[89–91] For example, DNA methylation status can be determined by direct PCR sequencing or cloning sequencing after treatment with sodium bisulfite which converts unmethylated cytosine residues to uracil whereas 5-methylcytosine (5mC) remains unaffected.[91]

With the advancement of next-generation sequencing (NGS) technologies, high-throughput methods capable of genome-wide and single-base resolution detection of epigenetic modifications have been developed (Figure 2A). Techniques such as whole-genome bisulfite sequencing (WGBS-seq),[92] reduced representation bisulfite sequencing (RRBS),[93] and methylated DNA immunoprecipitation sequencing (MeDIP-seq)[94] have revolutionized the profiling of DNA methylation. Chromatin immunoprecipitation sequencing (ChIP-Seq) deciphers genome-wide modification and distribution of histone proteins while RNA sequencing has enabled us to identify novel transcripts and better understand the diverse roles of ncRNAs including miRNA, siRNA, piRNA and lncRNA in gene regulation.[95,96] Additionally, assay for transposase-accessible

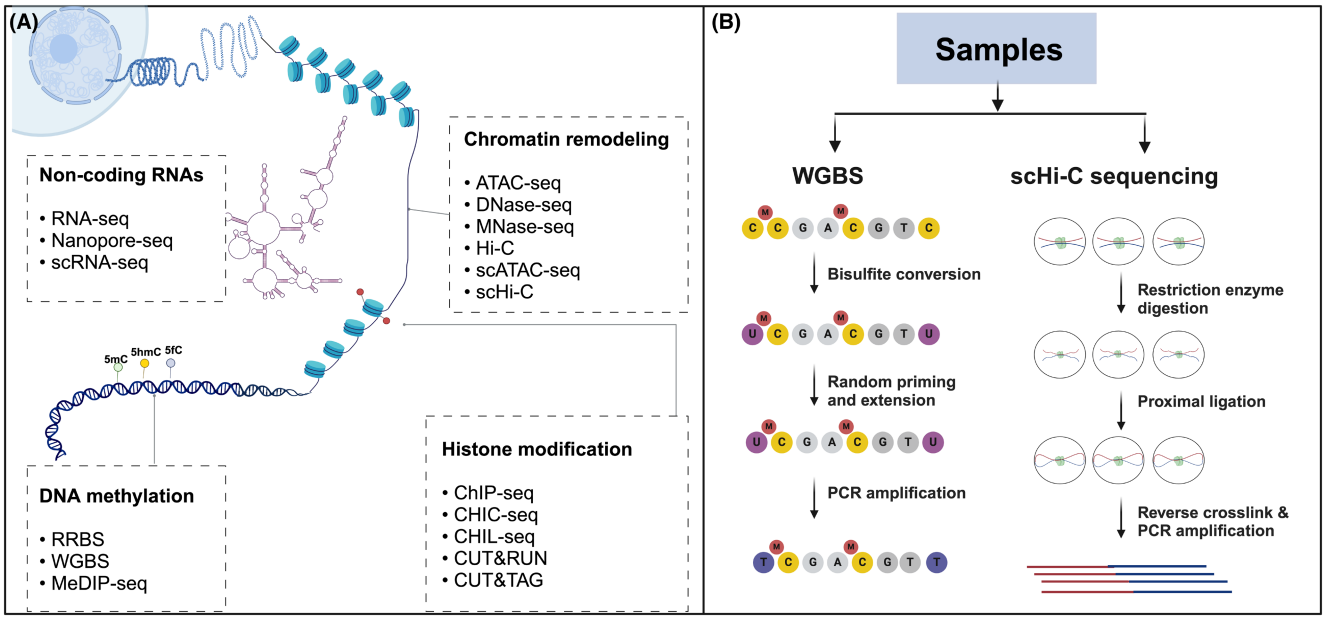


FIGURE 2 Overview of epigenomic techniques. (A) Epigenomic features and associated techniques: This panel summarizes key epigenomic features and the techniques used to study them. These include single-cell (sc) techniques, high-throughput chromosome conformation capture (Hi-C), chromatin immunoprecipitation followed by sequencing (ChIP-Seq), chromatin immunocleavage followed by sequencing (ChIC-Seq), chromatin integration labeling sequencing (ChIL-Seq), cleavage under targets and release using nuclease (CUT and RUN), cleavage under targets and tagmentation (CUT and TAG), assay for transposase-accessible chromatin sequencing (ATAC-Seq), DNase I hypersensitive site sequencing (DNase-Seq), micrococcal nuclease sequencing (MNase-Seq), reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing (WGBS-Seq). (B) Representative new techniques in CVD research: This panel highlights innovative techniques such as WGBS and scHi-C that are particularly useful in studying the epigenomic impacts of behavioral and environmental factors on CVD. WGBS integrates bisulfite conversion with next-generation sequencing (NGS) to provide a comprehensive methylation profile, while scHi-C combines single-cell resolution with Hi-C technology to map chromatin interactions at the single-cell level.

chromatin sequencing (ATAC-seq)[97] and DNase I hypersensitive site sequencing (DNase-seq)[98] assays measure chromatin accessibility based on enzymatic sensitivity, offering insights into chromatin remodeling under different physiological status. These methods have been extensively used to study methylation and histone modification patterns in genes associated with CVD.[99–102]

Recent technological advancements in three-dimensional (3D) genome organization enable researchers to delve into the intricate folding and organization of DNA within the nucleus, yielding valuable insights into chromatin structure, genome architecture, and the spatial interactions among distant genomic regions.[103] Techniques for analyzing 3D genome organization encompass sequencing-based methods like Hi-C and its derivatives,[104] microscopy-based approaches such as multiplex fluorescent in situ hybridization (FISH),[105] and computational modeling methodologies.[106] For instance, researchers identified noncoding genetic variants by performing epigenetic profiling of enhancer H3K27ac using ChIP-seq in 70 human control and end-stage failing hearts. Disease and phenotype associations have been found for 62 unique loci. These loci may exert their effects through alterations in enhancer H3K27-acetylation enrichment and the resulting differences in gene expression.[107]

Conventional bulk RNA sequencing (RNA-Seq) analysis which averages the epigenetic information of cell populations, is unable to resolve the heterogeneity within individual cells. Single-cell epigenomic analysis has the potential to overcome these limitations and to elucidate gene regulatory mechanisms within heterogeneous cell populations inside the heart (Figure 2B).[108] For example, single-cell reduced representation bisulfite sequencing (scRRBS) technique modified the original RRBS method by integrating all the experimental steps before PCR amplification into a single-tube reaction at a single cell resolution.[109] Additionally, single-cell whole-genome bisulfite sequencing (scWGBS) offers a genome-wide approach, providing comprehensive methylation profiles at single-cell resolution, making it a powerful tool for studying global epigenetic landscapes.[110] Single-cell sequencing assay for transposase-accessible chromatin (scATAC-Seq) is the state-of-the-art technology for analyzing genome-wide open chromatin profiles in single cells.[111] Single-cell Hi-C (scHiC) enables us to understand 3D chromatin organization in each nuclear within a heterogeneous cell population (Figure 2B).[112] Single-cell multi-omics integrate multiple omics techniques such as genomics, transcriptomics, epigenomics, proteomics at the single-cell level, allowing simultaneous analysis of various molecular characteristics within individual cells.[113] In addition, these descriptive techniques can be complemented by the versatile toolbox of genome engineering technologies such as clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR-Cas). It is possible to mutate the genome precisely, reversibly and at multiple sites simultaneously. Furthermore, one can tether proteins of interest to selected genomic positions to silence or activate genes, to induce reversible changes in 3D chromatin architecture and to visualize loci of interest in live imaging experiments.[114]

Rapid advances in long-read sequencing technologies, particularly with platforms like Oxford Nanopore Technologies (ONT), have resulted in significant improvements in accuracy, read length, and throughput for sequencing long DNA and RNA molecules.[115–117] Unlike short-read technologies, ONT's unique mechanism enables direct detection of base modifications through alterations in ionic current.[118] Coupled with refined bioinformatics tools, nanopore sequencing has become a cornerstone for investigating genomes, transcriptomes, epigenomes, and epitranscriptomes.[119–123] The extended read length of Nanopore sequencing has proven invaluable for characterizing lncRNAs, including their abundance and modification patterns. For instance, Ward et al. identified novel lncRNAs associated with cardiac ischemia using nanopore sequencing.[124] Furthermore, comparative studies between methylated RNA immunoprecipitation sequencing (MeRIP-seq) and nanopore differential RNA-Seq (dRNA-Seq) have demonstrated the latter's superiority in precise m^6A quantification and localization.[125] The versatility of nanopore sequencing is exemplified by its application in elucidating rRNA biogenesis and modification dynamics in archaea.[126] Additionally, Nano-tRNAseq offers a comprehensive approach for quantifying tRNA abundance and modifications.[127] To further advance our understanding of epitranscriptomics, integrating nanopore sequencing with single-cell technologies will be crucial for capturing high-resolution, cell type-specific epitranscriptomic profiles.[128]

However, epigenomic profiling at the single-cell level remains a significant technical challenge. Single-cell DNA methylation methods are particularly limited by low genomic coverage, capturing only a subset of the methylome.[102] For example, single-cell reduced representation bisulfite sequencing (scRRBS) interrogates approximately 10% of the genome. Moreover, the inherent inability of DNA polymerases to replicate epigenetic modifications presents a substantial barrier.[129] While recent advancements, such as single-cell Hi-C and scRRBS, have enabled the exploration of chromatin interactions and DNA methylation at the single-cell level, these methods are constrained by limited coverage, resolution, and substantial technical noise.[109,130] Despite the generation of large-scale single-cell sequencing datasets, computational challenges persist. The unique characteristics of single-cell

data, including low coverage, sparsity, high error rates, amplification bias, and allelic dropout, necessitate specialized computational tools distinct from those used in bulk sequencing analysis.[131] Overall, overcoming these technical and computational challenges is crucial for the advancement of single-cell epigenomic profiling and its application in understanding cellular heterogeneity and epigenetic regulation.

In summary, these advancements in approaches and technologies are revolutionizing the ways in which epigenetic processes can be studied, understood, and harnessed to understand physiological and pathological processes and to develop novel biomarker and therapeutic strategies.

4 | EPIGENETIC MECHANISMS MEDIATE BEHAVIORAL AND ENVIRONMENTAL RISK FACTORS-RELATED CVD

Risk factors for CVD include a diverse range of internal and external influences that contribute to the onset or exacerbation of cardiovascular conditions. These factors can be categorized into five groups: behavioral, physiological, demographic, environmental, and genetic.[132] Physiological, demographic, and genetic risk factors are nonmodifiable risk factors. The most important behavioral risk factors of CVD are unhealthy diet, physical inactivity, tobacco use, and harmful consumption of alcohol. Among environmental risk factors, air and noise pollution are important factors. In this review, we focused on one group of modifiable risk factors: behavioral risk factors (smoking, alcohol, physical activity, diet, and obesity). In addition, we discussed environmental risk factors (air and noise pollution). These seven risk factors account for an estimated 80% of CVD (Figure 3).[2]

4.1 | Smoking

While global smoking prevalence decreased from 22.7% in 2007 to 17% in 2023, smoking remains a major cause of CVD, estimated to account for more than 8 million deaths worldwide.[133] Smoking is known to damage vascular endothelium, which initiates atherosclerosis by first manifesting as endothelial dysfunction and later progressing into CVD. It is associated with abnormal nitric oxide (NO) production, inflammation, apoptosis, oxidative stress, aortic stiffness, increased blood pressure, decreased flow-mediated dilation, and cardiac autonomic imbalance toward sympathetic predominance—all contributing to increased CVD risk.[134] However, the molecular mechanism remains unclear, which barriers us from identifying an effective approach to proactively predict and prevent smoking-related CVD.

Cigarette smoke is an extremely complex aerosol that contains over 7000 compounds and at least 250 are known to be harmful to our health. Among these toxins, nicotine, carbon monoxide, benzene, arsenic and formaldehyde are the most toxic compounds inside cigarette smoke.[135] These chemicals can affect epigenetic modification by directly or indirectly suppressing epigenetic modifying enzymes via changing the availability of substrates required for those enzymatic reactions. Notably, epigenetic modifications, including DNA methylation and histone modifications, play crucial roles in mediating the effects of smoking on cardiovascular health.[136,137] For instance, high dose of arsenic exposure leads to the depletion of S-adenosylmethionine (SAM), the primary methyl donor in cells, consequently suppresses the expression of DNA methyltransferase genes such as DNMT1 and DNMT3A.[138] Cigarette smoke exposure is also associated with a decreased expression level of DNMT3B.[139,140] Previous studies have implicated hypomethylation of various genes, such as aryl hydrocarbon receptor repressor (*AHRR*), coagulation factor II receptor-like 3 gene (*F2RL3*) and protease-activated receptor-4 (*PAR4*) in increased smoking-related CVD risk. For *AHRR*, researchers found that hypomethylation at the cg05575921 site predicts the risk of myocardial infarction (MI), particularly in former smokers.[141] The AHRR and its receptor aryl hydrocarbon receptor (AHR) pathway is pivotal for detoxifying tobacco toxins.[142] Similarly, DNA hypomethylation at *F2RL3* is associated with 34% of smoking-induced pathological thrombosis via protease-activated receptor 4 (PAR4), which mediates the increased of platelet reactivity in myocardial infarction.[143] Another study reveals that cigarette smoke exposure decreases HDAC activity and reduces HDAC1, HDAC2, and HDAC3 expressions in macrophages, thereby leading to an inflammatory response.[144]

Chromatin remodeling is yet another epigenetic response to environmental stimuli. For example, nicotine exposure decreased expression of BAHCC1 and SMARCA2.[145] BAHCC1 is associated with chromatin transcription silencing.[146] SMARCA2, on the other hand, is a core ATPase subunit of the ATP-dependent chromatin remodeling complex SWI/SNF that mobilizes nucleosomes and plays key roles in gene transcription regulation, genome replication and repair during development.[147] These data suggested that BAHCC1 and SMARCA2 could be used as biomarkers for dysregulated chromatin remodeling in CVD in response to nicotine exposure.

ncRNAs, including microRNAs like miR-132 and miR-155, are emerging key players in the molecular response

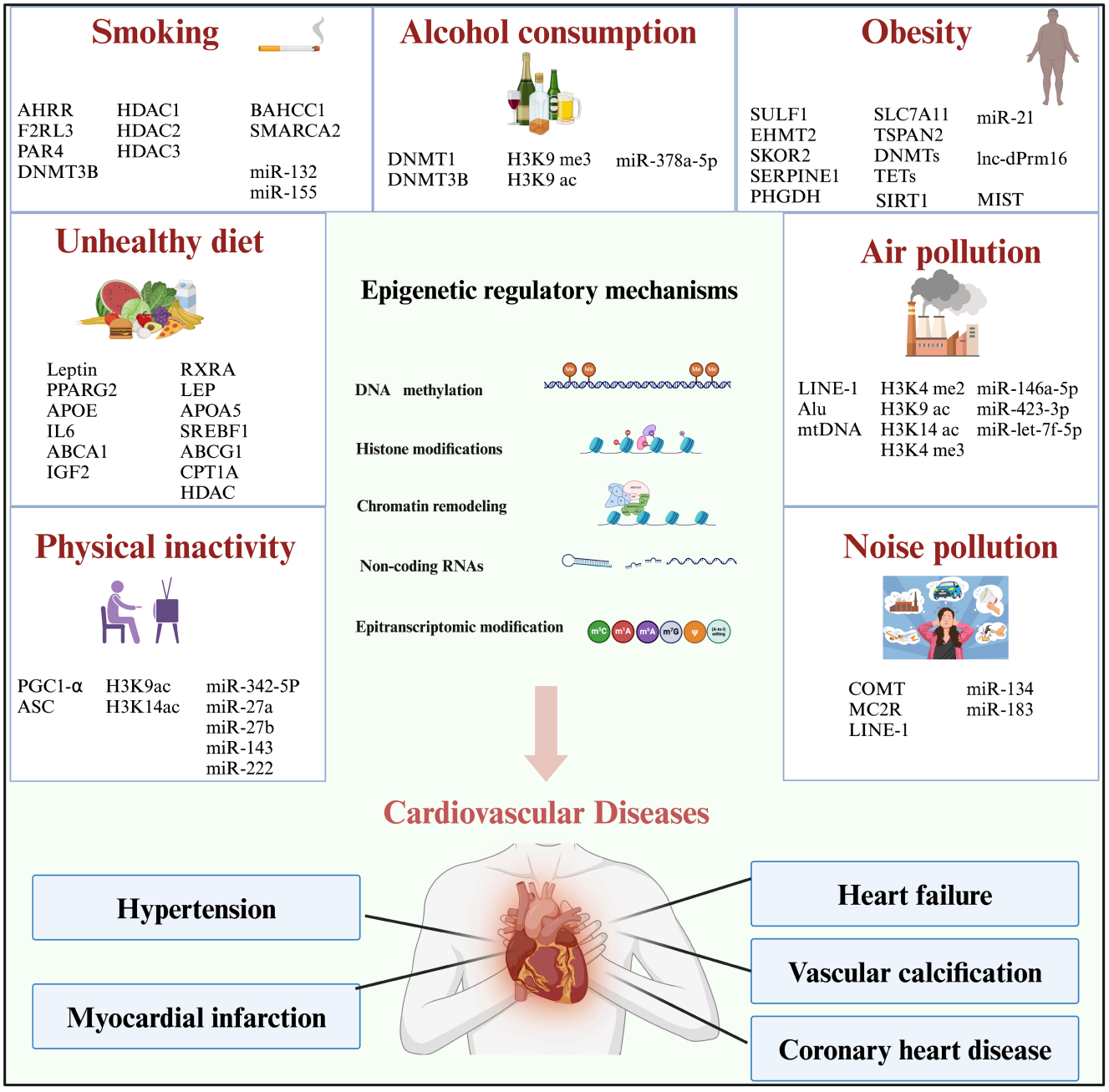


FIGURE 3 Overview of epigenomic mechanisms related to CVD induced by environmental and behavioral factors. Seven most modifiable environmental and behavioral factors including smoking, alcohol, diet, physical inactivity, obesity, air pollution and noise pollution-related epigenetic changes (DNA methylation, histone modifications, noncoding RNA (ncRNA) expression chromatin remodeling, and epitranscriptomic modification) in CVD. Representative epigenetic changes for each behavioral and environmental factor are listed in the box.

to smoking, influencing processes such as cardiomyocyte apoptosis, inflammation, and immune responses. Research suggests that miR-132 is upregulated in the serum of smokers compared with its expression in the nonsmoker controls, and miR-132 inhibits cardiomyocyte apoptosis so as to ameliorate myocardial remodeling in rats with MI through downregulation of inflammatory cytokines Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα).[137] IL-1β and TNFα are crucial factors for inflammation following MI via modulation of immune cell recruitment and cytokine production, and increased IL-1β and TNFα levels are adversely related to myocardial remodeling in MI. Another well-studied miRNA related to smoking is miR-155. It is upregulated in response to cigarette smoke exposure and has been implicated in the pathogenesis of smoking-related vascular disease such as atherosclerosis.[148,149]

4.2 | Alcohol consumption

Alcohol consumption is a well-recognized factor in CVD risk. Heavy drinkers, defined as individuals who consume four or five drinks or more per day, are associated with various CVDs including alcoholic cardiomyopathy, high blood pressure, increased risk of myocardial infarction, arrhythmias, fatal cardiac arrest and stroke.[150] In addition, recent studies have shown that alcohol consumption, even at moderate levels, is associated with adverse outcomes of cardiovascular health.[151] Alcohol can impact cellular activity and enzyme function directly, or it can indirectly damage the cardiovascular system through an early breakdown byproduct called acetaldehyde. Acetaldehyde typically remains in the body briefly before converting into acetate. Acetaldehyde, even as a short-lived intermediate product, can directly interact with DNA to form adducts and crosslinks, which can interfere with DNA replication and repair processes. This DNA damage can contribute to mutations and carcinogenesis. In addition, acetaldehyde can modify proteins through the formation of adducts, leading to altered protein structure and function. This can disrupt cellular processes and contribute to cellular dysfunction.[152] Evidence indicates that alcohol can influence epigenetic modifications. Kutay et al. found that alcohol affects DNA methylation patterns by reducing the protein levels and activities of key DNA methylation enzymes, DNMT1 and DNMT3B. Similar to tobacco smoke, alcohol also affects the activities of HATs and HDACs that mediate histone acetylation and deacetylation.[153]

Maternal alcohol consumption during gestation has also been shown to be closely related to the occurrence of congenital heart disease. A recent study indicated that alcohol caused histone H3K9me3 hypomethylation by altering histone methyltransferase G9α expression in cardiomyocytes.[154] The expression of key cardiomyogenesis genes, *Mef2c*, *Cx43*, *Nppa*, and *β-MHC* were downregulated in alcohol-exposed fetal mouse hearts, suggesting that aberrant level of H3K9me3 mediated by G9α may account for the alcohol-induced abnormal expression of cardiomyogenesis-related genes during pregnancy. The authors further found that alcohol increased H3K9 acetylation (H3K9ac) and the expression of the transcription factors including GATA4 and MEF2C in cardiac progenitor cells. In addition, large number of miRNAs have been identified to be altered post ethanol consumption. For example, one study showed that a substantial elevation of miR-378a-5p in acute ethanol exposure of rat cardiomyocytes, highlighting the potential impact of alcohol consumption on cardiac transcriptome and epigenetics regulation.[155]

4.3 | Physical inactivity

Physical inactivity has been shown to be a significant risk factor for CVD. It ranks similarly to cigarette smoking. Numerous studies have documented an inverse relationship between physical activity (self-reported or objectively measured) and CVD as well as all-cause mortality.[156] It has been shown that exercise can directly modify cardiac epigenetics to promote cardiac health and to protect the heart against various pathological processes. Further, exercise can modify epigenetics in other tissues, which reduces the risk of cardiac disease and offers cardioprotection through organ cross-talk.[157] For example, Hou et al. found that exercise-induced exosomal miR-342-5p secretion activates cardiac survival signaling, which decreases cardiac vulnerability in myocardial ischemia/reperfusion injury.[158]

Notably, physical inactivity has been linked with hypomethylation of the *PGC1-α*, a key transcriptional coactivator gene for mitochondrial biogenesis and fatty acid oxidation in cardiac muscle, suggesting hypermethylation of *PGC-1α* as a key biomarker for physical inactivity-related CVD such as heart failure.[159] Exercise increases the methylation of the adaptor protein, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), which is necessary for inflammasome activation of IL-1β. ASC with higher methylation and lower expression is positively related to the outcome of heart failure.[160] In addition, it has been reported that exercise-modulated DNA methylation in other tissues prevents inflammation and remodels metabolism, consequently reduces the incidence of cardiac diseases.[161] Some studies have also suggested that histone modifications induced by exercise can alleviate cardiac disease risk through metabolic remodeling. For instance, researchers in a study using a rat swimming model observed an increase in H3K9/14 acetylation, which regulates *GLUT4* at transcriptional level through action on its promoter,[162] further suggesting a protective role of exercise in heart.

The alterations in ncRNA induced by exercise also constitute a large part of epigenetic modification after exercise. Numerous miRNAs in the heart are altered by exercise and have been shown to be involved in many biological processes, including angiogenesis, cardiac physiological hypertrophy, and cardiomyocyte survival.[163] For instance, aerobic exercise induced cardiac hypertrophy in rats through regulation of cardiac miRNAs, including miR-27a, miR-27b, and miR-143, which modify renin-angiotensin system genes posttranscriptionally.[163] In hearts of healthy subjects, exercise modulates the expression of miR-222, which mainly regulates cardiomyocyte growth and left ventricular hypertrophy.[164]

The epitranscriptome remodeling has been tightly associated with physical activity. Studies showed that excerise could change the expression level of key RNA modification enzymes, including writing enzymes, reading enzymes, and erasing enzymes.[165–167] Endurance exercise training has been previously demonstrated to reduce cardiac mRNA m^6A levels. This decrease of RNA modification level appears to be an outcome for the downregulation of RNA methylation writer METTL14 post exercise, and subsequently, expression of METTL14 can block exercise-induced physiological cardiac hypertrophy.[166] Another study has implied that exercise could mitigate endothelial pyroptosis and atheroscleosis through downregulating lncRNA NEAT1 through a m^6A-dependent mechanism.[168]

4.4 | Unhealthy diet

It is estimated that dietary risk factors are associated with 53% of CVD deaths, in part as a result of its effects on other major CVD risk factors.[169] Among different dietary patterns, dietary patterns rich in fruits and vegetables as well as the Mediterranean diet (MedDiet) rich in olive oil, legumes, fruits, and vegetables, have been associated with lower CVD incidence and mortality, including heart failure and myocardial infarction.[170] MedDiet is associated with increased DNA methylation and thus suppression of the expression of genes related to inflammation and immunocompetence.[171] Dietary components including macronutrients, folic acid and other bioactive compounds, are used to produce metabolites that can impact gene expression directly or through epigenetic mechanisms.

Macronutrients—carbohydrates, fat, and protein are called macronutrients. Excess carbohydrate and lipid elevate acetyl-CoA, and can change chromatin structure, increase acetylation of DNA-binding proteins, suppress autophagy, and accelerate age-associated pathologies.[172] Circulating fatty acids such as α-linolenic acid, EPA and DHA affect changes in DNA methylation sites for genes such as APOE, IL6 and ABCA1 which are correlated with CVD traits.[173] Obesogenic high-fat diets were associated with increased DNA methylation in the Leptin and *PPARG2* gene promoters.[174] Several cohort studies showed that a high-fat dietary challenge increased DNA methylation at sites of genes such as *APOA5*, *SREBF1* and *ABCG1* that regulate lipogenesis and lipoprotein metabolism, while it lowered methylation of CPT1A which promotes hepatic fatty acid β-oxidation.[175–177]

Folic acid, *B-vitamins*, *other methyl donors*—among the compounds identified for their ability to influence DNA methylation, particular attention is given to nutrients involved in folate-mediated one-carbon metabolism. Key nutrients in this pathway include folate, methionine, and various B vitamins (such as B2, B6, and B12), which serve as essential cofactors for one-carbon transfer reactions. This pathway is vital for generating SAM, the universal methyl donor in cellular reactions. Other methyl donors, such as choline and betaine, can also affect the SAM status, primarily through choline-mediated one-carbon metabolism, and ultimately impact DNA methylation.[178] Pauwels et al. reported that maternal dietary and supplemental intake of methyl-group donors, in the periconception period only, increased infant buccal DNA methylation in genes related to growth (*IGF2*), metabolism (*RXRA*), and appetite control (*LEP*), all could affect cardiovascular health.[179] Biotin, an essential water-soluble B vitamin, affects histone tails H2A, H3, and H4 by covalently attaching biotin to certain lysine residues that are catalyzed by the enzymatic reactions involving biotinidase and holocarboxylase synthetase. Biotinylation reactions specifically at lysine 8 and lysine 12 in histone H4 have been implicated in heterochromatin structure, gene inactivation, mitotic chromatin condensation, and DNA repair.[180]

Bioactive compounds—polyphenols (flavonoids, curcuminoids, and stilbenes) contained in fruits, vegetables, and other dietary components (green tea, red wine, cocoa, etc.) compose the largest group of bioactive compounds with well-documented anti-inflammatory and cardioprotective actions.[181] These compounds act as weak ligands for HDAC and exhibit HDAC inhibitory activity.[182] For example, in a human study, broccoli sprouts (68 g) inhibited HDAC activity and activated histone H3 and H4 acetylation in circulating peripheral blood mononuclear cells.[183] Curcumin, a bright yellow chemical compound produced by plants of the Curcuma longa species inhibits p300 histone acetyltransferase activity and prevents heart failure.[184] Resveratrol, butyrate, sulforaphane, and diallyl sulfide inhibit HDAC activity.[185] Altered enzyme activity by these compounds may affect physiologic and pathologic processes during our lifetime by altering gene expression.

4.5 | Obesity

Obesity prevalence continues to escalate rapidly in many regions worldwide, with projections indicating that by 2025, it will exceed 18% in men and surpass 21% in women globally.[186] It is a significant risk factor for a broad spectrum of CVD including coronary heart disease, heart failure, hypertension, stroke, atrial fibrillation, and sudden cardiac death. Obesity can directly induce structural and functional adaptations of the cardiovascular system to accommodate excess body weight, as well as by adipokine effects on inflammation and vascular homeostasis.[187]

Obesity is also associated with altered epigenetic patterns including histone modifications, DNA methylation and hydroxymethylation, and miRNA expression.[188]

A systematic review of the role of DNA methylation in modifying blood pressure showed that lower methylation levels of sulfate endosulfatase (*SULF1*), euchromatic histone lysine methyltransferase 2 (*EHMT2*, also known as *G9a*), and SKI family transcriptional corepressor 2 (*SKOR2*) were associated with hypertension related to obesity. On the other hand, lower methylation levels of phosphoglycerate dehydrogenase (*PHGDH*), Solute Carrier Family 7 Member 11 (*SLC7A11*), and tetraspanin 2 (*TSPAN2*) were correlated with higher systolic and diastolic blood pressure.[189] Imbalances in the activity of enzymes responsible for methylation and demethylation, such as DNMTs and TETs are observed in obesity and may offer new avenues for diagnostic approaches and therapeutic interventions.[190]

Changes in histone acetylation caused by high-fat diets have been linked to obesity. Sirtuin 1 (SIRT1) is a member of a Class III HDACs dependent on nicotinamide adenine dinucleotide (NAD+). Studies have shown that SIRT1 is a novel central modulator of the earliest microvascular damage induced by obesity. Through a complex epigenetic control mainly involving $p66^{Shc}$ and arginase II, SIRT1 influences mitochondrial reactive oxygen species (mtROS) levels, NO availability, and the expression of proteins of the mitochondrial respiratory chain.[191] Early targeting of SIRT1 might represent a crucial strategy to prevent obesity-related microvascular dysfunction.

miRNAs and lncRNAs have also been implicated in the pathogenesis of obesity-induced CVD. For example, an induced expression of miR-21 was found in the white adipose tissues of obese people compared to lean controls and correlated with impaired vascular function.[192] lncRNAs, such as *lnc-dPrm16* and *MIST*, have both been shown to influence brown adipogenesis, inflammation, and lipid metabolism.[193] Obesity and diabetes could also regulate the epitranscriptome remodeling, especially through regulating the m^6 A methylation eraser enzyme fat mass and obesity-associated protein (FTO). FTO is the first reported m^6A demethylase and it was initially being identified by Genome-Wide Association Studies (GWAS) that a single-nucleotide polymorphisms (SNP) of the FTO gene is associated with obesity.[194] Mice studies have shown that increased expression of FTO causes obesity post high-fat diet challenge.[195] Another study investigating the m^6A writing enzyme-METTL3's role in obesity has further implied that METTL3 plays an important role in macrophage metabolic reprogramming in obesity potentially through changing m^6A modification level of DNA damage inducible transcript 4 (DDIT4) mRNA, highlighting the involvement of RNA modification in obesity.[196]

4.6 | Air pollution

Air pollution is a well-recognized major risk factor for CVD and has been estimated to contribute more to global morbidity and mortality than all other known environmental risk factors combined. Although air pollution contains a heterogeneous mixture of gases, the most detrimental to health include fine particulate matter (particles ≤2.5 μm in diameter, $PM_{2.5}$) and ozone gas.[197] A short-term inhalation of $PM_{2.5}$ has been shown to elevate the levels of cytokines in promoting inflammation, coagulation, and vasoconstriction, subsequently lead to airway hyper-reactivity, reduced lung function, inflammation, thrombosis, high blood pressure, atherosclerosis, and ischemia.[198,199] It was found that exposure to $PM_{2.5}$, CO, and O_3 altered the methylation pattern in many CpG sites for the immunoregulatory genes *Foxp3*, *IL-4*, *IL-10*, and *IFN-g*, thereby altering the immune response.[200] Epidemiological studies reveal that $PM_{2.5}$ and PM_{10} exposures induce the hypomethylation of *Alu* and/or *LINE1* (long interspersed nuclear element-1) elements in leukocytes and buccal cells.[201] Demethylation of *LINE-1* repetitive elements was correlated with greater vascular cell adhesion molecule 1 (VCAM-1) expression in serum, especially in CVD-free participants, indicating that the activation of LINE-1 hypomethylation–VCAM-1 axis occurred in the initial phase of CVD.[174] In addition to nuclear DNA, ambient pollutants can also impact mitochondrial DNA (mtDNA). In a study comprising 48 healthy male workers without known CVD, blood mtDNA methylation in the D-loop promoter was inversely correlated with $PM_{2.5}$ concentrations. Notably, subjects displaying superior mtDNA methylation levels were also prone to the effect of $PM_{2.5}$ exposure induced heart rate variability.[202]

In addition to changing the DNA methylation status, air pollution could also impact on histone modification. One study shows that inhaled nickel, arsenic, and iron are associated with increased H3K4Me2 and H3K9Ac on histones in human blood leukocytes.[203] PAH exposure also induces histone modifications in mice—for instance, exposure to benzo[a]pyrene (BaP), a type of PAH, decreases the acetylation of H3K14 in the steroidogenic acute regulatory protein (StAR) gene promoter, which is important for testosterone production. BaP exposure to HeLa cells induces H3K4Me3 and H3K9Ac modifications along with a reduced association of DNMT1 with the *LINE-1* promoter.[204]

Recent studies have found that $PM_{2.5}$ can affect the expression of miRNAs with potential biological effects.[205] For example, $PM_{2.5}$ exposure induces expression of miR-146a-5p, miR-423-3p, and miR-let-7f-5p in regulatory T cells of acute MI patients, indicating miRNAs could be biomarkers for $PM_{2.5}$ exposure-related acute MI.[206] In

addition, air pollution could also affect the RNA modification. According to a study characterizing the changes in m^6A modification from peripheral blood collected from Beijing Truck Driver who are being exposed to air pollution, Kupsco et al. have shown m^6A is negatively associated with long-term smoking, yet positively associated with short-term personal black carbon (BC) exposure, pointing to m^6A being used as a potential biomarker for environmental health research.[207]

4.7 | Noise pollution

Noise pollution, like air pollution, has significant adverse effects on human health, including various non-auditory impacts such as reduced well-being, impaired communication, decreased mental concentration, and emotional stress, contributing to noise-induced annoyance.[208–210] Recent studies have increasingly linked noise pollution to CVDs.[211] For example, prolonged exposure to continuous noise (80–100 decibels) in animal models has been shown to elevate heart rate and systemic arterial blood pressure, accompanied by increased plasma levels of corticosterone, adrenaline, and endothelin-1.[212] A meta-analysis of 14 studies found a pooled relative risk of 1.08 for coronary artery disease (CAD) associated with road traffic noise, with an 8% increase in CHD risk for every 10 decibels A (dBA) rise in noise levels between 52 and 77 dBA day-night average sound level (Ldn).[213] Additionally, noise exposure in this range is linked to higher hypertension rates, particularly among men, older adults, and diabetics, and similar associations with stroke have been observed.[214] Greater exposure, such as that near major noise sources like airports, shows more pronounced effects. Individuals living near airports have an elevated risk of arterial hypertension, CHD, stroke, and CVD hospitalization.[214]

Recent research has also explored the epigenetic mechanisms underlying noise pollution's impact. A study found that short-term noise exposure was associated with altered DNA methylation of the catechol-O-methyltransferase (*COMT*) gene in the rat medulla oblongata. Long-term nighttime noise exposure led to abnormal methylation of *COMT*, melanocortin 2 receptor (*MC2R*), and *LINE-1* regions in the rat brain, correlating with changes in blood pressure and body weight.[215] Changes in DNA methylation in response to noise exposure suggest an epigenetic regulation of metabolic processes, particularly those involving stress hormones.[215] Additionally, noise-induced stress affects ncRNA expression, including microRNAs such as miR-134 and miR-183, which are upregulated in response to stress and are associated with CAD and depression.[216]

5 | EPIGENETIC BIOMARKES FOR BEHAVIORAL AND ENVIRONMENTAL FACTORS-RELATED CVD

Epigenetic modifications are highly dynamic and responsive to environmental and lifestyle factors in a tissue-specific manner. This dynamicity allows for the detection of real-time changes in gene expression patterns, making epigenetic biomarkers valuable for monitoring disease progression tailored to specific diseases or organ systems over time.[6] In addition, epigenetic changes often occur early in disease development, even before clinical symptoms manifest.[217] These unique characteristics of epigenetics, including its dynamic nature and tissue specificity, make it a powerful tool for biomarker discovery and personalized medicine applications. Here, we explored the role of epigenetics serving as biomarkers for CVD linked to environmental factors. We have compiled a selection of significant biomarkers showing promising links to CVD risk, detailed in Table 1. These insights serve as valuable references for researchers and clinicians.

Recent research on *AHRR* hypomethylation at the cg05575921 site is associated with increased risk of smoking-related coronary heart disease status. Specific analysis of *F2RL3* identified a single locus, cg03636183, which had significantly lower genome-wide methylation in smokers. These data suggest hypomethylation at these two specific sites could serve as a biomarker for researchers to further examine in smoking-related CVD.[141,143,218,219] H3K9me3 hypomethylation could be a biomarker for maternal alcohol consumption-associated congenital heart disease.[154] Moreover, investigations into the DNA methylation patterns of the *SERPINE1* gene revealed associations with increased risk of metabolic syndrome associated with obesity.[220] Other research studying the epigenetics of obesity found that hypermethylation of the *LEP* gene was negatively associated with obese subjects weight.[221] Hypermethylation of *PGC-1α* is a key biomarker for physical inactivity-related CVD such as heart failure.[159] Maternal dietary and supplemental intake of methyl-group donors in the periconception period, increased infant buccal DNA methylation in genes related to *IGF2*, *RXRA*, and *LEP*.[179] Circulating fatty acids such as α-linolenic acid, EPA, and DHA affect DNA methylation status for genes such as *APOE*, *IL6*, and *ABCA1* which are correlated with CVD traits.[173] Obesogenic high-fat diets were associated with increased DNA methylation in the leptin and PPARG2 gene promoters.[174] Research on air pollution led to the finding that prolonged exposure to black carbon (BC), and sulfates (SO_4) was associated with hypomethylation of two types of repetitive elements, *Alu* and *LINE-1*.[201,222] One study suggested that blood mtDNA

TABLE 1 List of epigenomic biomarkers related to CVD induced by behavioral and environmental factors.

Epigenetic modification	Biomarker	Environmental factors	Disease type	Study type	Reference
DNA methylation	AHRR (cg05575921)	Smoking	Myocardial infarction	Human cohort studies	[141]
	F2RL3 (cg03636183)	Smoking	Coronary heart disease, atherosclerosis	Human cohort studies	[143]
	Hypermethylation of PGC-1α	Physical inactivity	Heart failure	Animal study	[159]
	APOE, IL6, ABCA1, Leptin and PPARG2	Unhealthy diet	CVD	Human cohort studies	[173]
	IGF2, RXRA, and LEP	Lack of folic acid in maternal dietary	CVD	Human cohort study	[179]
	PHGDH, SLC7A11, TSPAN2	Obesity	Metabolic syndrome	Human cohort study	[189]
	SULF1, EHMT2, and SKOR2	Obesity	Hypertension	Human cohort study	[189]
	Alu and LINE-1 repetitive elements	Black carbon and sulfates in air pollution	Early stage of CVD	Human cohort study	[201]
	mt DNA methylation	Air pollution	CVD	Human cohort study	[202]
	COMT, MC2R, LINE-1	Noise pollution	Blood pressure	Animal study	[215]
Histone modification	H3K9me3 hypomethylation	Maternal alcohol consumption	Congenital heart disease	Animal study	[154]
Noncoding RNA	miR-132	Smoking	Myocardial infarction	Animal study	[137]
	miR-155	Smoking	Atherosclerosis	Cellular study	[148,149]
	miR-21	Nicotine	Abdominal aortic aneurysms	Animal study	[192]
	lnc-dPrm16	Obesity	Inflammation, lipid metabolism	Animal study	[193]
	MIST	Obesity	Inflammation, lipid metabolism	Animal study	[193]
	miR-27a, miR-27b, miR-222 and miR-143	Exercise	Cardiac hypertrophy	Animal study	[163,164]
	miR-21	Obesity	Impaired vascular function	Human and animal studies	[192]
	miR-146a-5p, miR-423-3p, and miR-let-7f-5p	PM2.5	Acute myocardial infarction	Human cohort study	[206]
	miR-134, miR-183	Noise pollution	CVD and depression	Animal study	[216]
Epitranscriptomic modification	m^6A (lncRNA NEAT1)	Physical inactivity	Atherosclerosis	Animal study	[168]
	m^6A (DDIT4)	Obesity	Metabolic syndrome	Animal study	[196]

methylation in the D-loop promoter was inversely correlated with $PM_{2.5}$ concentrations.[202] In summary, these DNA methylation and histone modification biomarkers, spanning various lifestyle and environmental factors, offer promising avenues for risk assessment, prognosis, and targeted interventions in the management of CVD.

In addition, miRNAs have been implicated in environmental factors-related CVD, and are speculated to initiate quicker, more dynamic gene expression changes in response to environmental changes. For instance, miR-155 is significantly associated with smoking-related vascular disease such as atherosclerosis.[148,149] miR-27a, miR-27b, miR-143, and miR-222 are associated with aerobic exercise-induced cardiac hypertrophy.[163,164] miR-21 is associated with obesity-related vascular dysfunction.[192] Recent studies further showed that $PM_{2.5}$ exposure induces miR-146a-5p, miR-423-3p, and miR-let-7f-5p expression in regulatory T cells of acute MI patients, indicating that miRNAs could be biomarkers for $PM_{2.5}$ exposure-related acute MI.[206] Collectively, these findings present a comprehensive framework for our current understanding of epigenetics in relation to CVD biomarkers and offer promising directions for future research and clinical interventions.

6 | EPIGENETIC THERAPEUTICS FOR ENVIRONMENTAL FACTOR-RELATED CVD

Understanding the epigenetic landscape associated with CVD risk factors opens avenues for targeted interventions aimed at mitigating disease progression and improving patient outcomes. Approaches targeting DNA methylation, histone modification, ncRNA and chromatin modifiers can lead to individualized precision medicine therapies in the future. In addition, epigenetic modifications are reversible. This offers an optimistic prospect for the treatment of CVD using epigenetic modifiers or related drugs to regulate target genes methylation status and expression level. For example, one animal study showed that DNMT inhibitor 5-aza-2′-deoxycytidine (5aza2DC) attenuates glucocorticoid-mediated fetal programming of blood pressure.[223] 5-Azacytidine is a nucleoside analog that inhibits its DNA methyltransferases (DNMTs), leading to global DNA hypomethylation.[224] While effective for certain cancers, its lack of specificity poses challenges for cardiovascular diseases, potentially causing off-target effects by activating unrelated genes. Currently, histone deacetylase inhibitors (HDACi) such as vorinostat and romidepsin are used in cancer treatment, but their application in CVD is limited due to nonspecific gene activation or suppression.[4] Thus, there is a need for histone modification-related drugs with high specificity and low side effects for cardiovascular diseases.

According to recent research findings, histone methyltransferase inhibitors or HAT/HDAC inhibitors are still rarely used in clinical treatment of CVD. However, the development of drugs targeting histone methylation and histone acetylation has achieved some effect in basic experimental research on the treatment of CVD. The histone H3K9 methyltransferase inhibitor chaetocin prolongs survival and restored mitochondrial dysfunction in a chronic heart failure model of Dahl salt-sensitive rats.[225] Resveratrol inhibits H3K27 methylation of vessels and blood biomarker and prevents deoxycorticosterone acetate (DOCA)-induced salt-sensitive hypertension. Resveratrol also improves cigarette smoke-induced endothelial dysfunction by activating a histone acetylase sirtuin 1.[226,227] Early targeting of SIRT1 by SIRT1 agonist (SRT1720) prevents age- and obesity-related microvascular dysfunction, suggest SRT1720 might represent a crucial strategy for vascular disease.[191] Another group demonstrated the protective effect of a substance in turmeric, curcumin on alcohol-induced cardiac damage during pregnancy.[228] HDAC inhibitors, trichostatin A and Statins can improve cigarette smoke-induced atherosclerosis and alcohol-induced alcoholic cardiomyopathy respectively.[229,230]

In recent years, increasing evidence has accumulated for noncoding RNAs function in gene regulation and CVD pathogenesis. Noncoding RNAs are attractive targets for potential clinical interventions. Currently, the field of nucleotide gene therapy, including antisense oligonucleotide (ASO) and siRNA, is developing rapidly. Analogs or inhibitors of noncoding RNA are easy to synthesize and have low cytotoxicity when transfected in vivo. AAV1 delivery of ncRNAs could also be applied for prevention and therapeutics. For example, AAV1 gene transfer of KLF4-shRNA could prevent and ameliorate the progression of cigarette smoke-induced pulmonary hypertension.[231] circRNA MFACR promotes cardiomyocyte death in MI and reduces miR-125b expression via methylation of the miR-125b gene. Overexpression of miR-125b reversed the effects of MFACR-mediated cardiomyocyte apoptosis,[232] suggesting both miR-125b and MFACR could be targets for hypoxia-induced MI. miRNA-181c could be a target for cigarette smoke-induced chronic obstructive pulmonary disease (COPD) by regulating CCN1, a key regulator in angiogenesis.[233] Extracellular vesicles (EVs) containing miRNAs could alleviate obesity-related metabolic dysfunction.[234] An animal study suggests that systemically administering miR-378 serves as a promising agent for preventing and treating obesity-related CVD in humans.[235] Similarly, miRNA-22-3p inhibition by miR-22-3p antagomir drug candidate APT-110 could be a potent treatment of fat accumulation, insulin resistance, and related complex

TABLE 2 List of potential epigenetic therapies related to CVD induced by behavioral and environmental factors.

Epigenetic classification	Drugs	Type	Targeted genes	Environmental factor	Diseases	Study type	References
DNA methylation	5-aza-2′-deoxycytidine	DNMT inhibitor		Glucocorticoid	Hypertension	Animal study	[223]
Histone methylation	Chaetocin	Histone H3K9 methyltransferase inhibitor	Mitochondria-related genes	High salt diet	Chronic heart failure	Animal study	[225]
	Resveratrol	Histone methylation-related drugs	H3K27me3	High salt diet	Hypertension	Animal study	[227]
Histone acetylation	Resveratrol	Sirtuin 1 natural agonist	Sirtuin 1	Cigarette smoke	Endothelial dysfunction	Cellular study	[226]
	Statins	HDAC inhibitor	HDAC1, HDAC2	Cigarette smoke	CVD	Meta-analysis	[229]
	Trichostatin A	HDAC inhibitor	HDAC class I and II	Alcohol	Alcoholic cardiomyopathy	Animal study	[230]
	SRT1720	SIRT1 agonist	SIRT1	Obesity	Microvascular Dysfunction	Cellular study	[191]
	Curcumin	HATs inhibitor	p300	Alcohol	Congenital heart disease	Cellular study	[228]
Noncoding RNAs	circRNA MFACR	circRNA	miRNA-125b	Hypoxia	Myocardial infarction	Cellular and animal studies	[232]
	miRNA-181c	miRNA-181c	CCN1	Cigarette smoke	Chronic obstructive pulmonary disease	Animal study	[233]
	AAV1-KLF4-shRNA	shRNA	KLF4	Cigarette smoke	Pulmonary hypertension	Animal study	[231]
	EV-miRNAs	EV-miRNAs	Fatty acid and cholesterol biosynthesis	Obesity	Metabolic dysfunction	Animal study	[234]
	miR-378	miR-378	Glucose metabolism	Obesity	CVD	Animal study	[235]
	APT-110	miR-22-3p antagomir	miRNA-22-3p	Obesity	Metabolic disorders	Animal study	[236]

metabolic disorders including obesity.[236] Given the above evidence from translational studies, drugs targeting epigenetics mechanisms are expected to be increasingly applied in clinical trials in the near future, in order to better meet the needs of ameliorating CVD patients' symptoms and improving prognosis (Table 2).

The potential of epigenetic drugs in treating CVD is immense, promising revolutionary therapeutic strategies.[4] However, significant challenges need to be addressed to realize this potential fully. One of the foremost challenges is ensuring the specificity of these drugs. It is crucial to design epigenetic drugs that precisely target the intended genes without causing unintended side effects.[4] Effective delivery of these drugs to the target cells and tissues also remains complex.[237] Additionally, optimal therapeutic outcomes may require combining epigenetic drugs with other treatments.[4]

7 | CONCLUSION AND OPEN QUESTIONS

In this review, we summarize recent data on epigenetic mechanisms and their regulatory roles in various behavioral and environmental factors-induced CVD. Studies have demonstrated that epigenetic markers associated with certain behavioral and environmental factors-induced CVD could be utilized for CVD diagnosis, and targeting epigenetic mechanisms holds promise for treating these diseases. With advancements in high-throughput sequencing technologies paving the way for personalized medicine approaches in CVD management, integrating epigenetic biomarker profiles with traditional clinical risk factors may lead to advancements in precision medicine and facilitate the delivery of tailored therapeutic interventions. This integration represents a paradigm shift in cardiovascular medicine, offering novel insights into disease pathogenesis and therapeutic avenues for intervention. Nonetheless, several challenges remain in the application of epigenetics in CVD.

First, establishing a causal link between different forms of epigenetic regulation and CVD pathogenesis remains challenging. While many studies have described associations between epigenetic differences induced by environmental factors using correlation studies, it is unclear whether these epigenetic changes directly cause CVD. New techniques combining epigenetics engineering and molecular tools are needed to manipulate epigenetic regulation. For instance, a combination of CRISPRi/a with induced pluripotent stem cells (iPSCs) could serve as an ideal platform for systematically identifying the causality of epigenetic regulation and environmental factors-induced CVD, facilitating biomarker discovery and drug development.[238] In addition, large multicenter studies are needed to provide convincing evidence for clinical applicability.

Second, many environmental exposures involve mixtures of chemicals as well as mixed lifestyles (i.e., physical inactivity plus alcohol consumption). With advances in mass spectrometry and other detection technologies, hundreds of chemicals and metabolites can now be measured simultaneously with high accuracy.[239] The effects of some toxic components and dynamics in metabolites on epigenetic modifications may be masked by other factors. Therefore, systematically profiling and defining causal chemicals/metabolites inside the environmental factors-induced epigenetic dysregulation in CVD is essential.

Third, most CVD are polygenic diseases resulting from the cumulative inheritance of multiple genetic variants alongside interactions with environmental factors. Given that most polygenic variants are influenced by environmental factors, it is essential to consider how environmental factors modulate genetic susceptibility in CVD. Future research directions could involve applying a village approach to culture human iPSCs and expose them to environmental risk factors, combined with single-cell omics to decipher genetic and environmental interactions induced CVD via epigenetic modifications.[240]

Overall, understanding the multidimensional "interactome" encompassing genetics, epigenetics, transcriptomics, proteomics, metabolomics, bioinformatics, demographic informatics, exposomics, and the entire life course is crucial for determining the health and disease outcomes of an individual. Continued research into environmental epigenetics and its implications on human health and disease variability is crucial for the development of personalized medicine and targeted therapies for CVD. This involves identifying novel epigenetic targets for therapeutic intervention, developing epigenetic-based diagnostic tests and prognostic markers, and integrating epigenetic profiling into personalized medicine approaches. Despite the challenges, integrating epigenetics into precision medicine for CVD holds great promise for revolutionizing modern medicine and improving patient outcomes.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Stored in repository.

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