EPIGENOMICS
2025, VOL. 17, NO. 14, 993–1008
https://doi.org/10.1080/17501911.2025.2540273

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REVIEW

Novel approaches and applications in identifying DNA methylation markers of cardio-kidney-metabolic disease

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ABSTRACT

Cardio-kidney-metabolic (CKM) diseases represent a major public health challenge, accounting for a large proportion of global burden of morbidity and mortality. These conditions share risk factors, including genetic predisposition, environmental exposures, and lifestyle influences, which collectively drive disease development and progression. Epigenetic modifications, particularly DNA methylation (DNAm), serve as key mediators and biomarkers between these risk factors and disease phenotypes by regulating gene expression without altering the DNA sequence. Epigenome-wide association studies have identified DNAm markers associated with CKM diseases and related phenotypes, highlighting both shared pathways and disease-specific epigenetic signatures in inflammation, metabolic dysfunction, and aging-related processes. Longitudinal studies further demonstrate the dynamic nature of DNAm changes over time, offering insights into disease trajectories. Additionally, methylation risk scores integrating multiple epigenetic markers show promise in improving disease prediction and risk stratification beyond traditional clinical factors. To synthesize the current evidence, we conducted a targeted literature search in PubMed for English-language, peer-reviewed articles published between 2014 and the present. Future research leveraging large, well-phenotyped cohorts, advanced statistical methods, and innovative study designs will be critical for uncovering novel biomarkers, refining risk prediction models, and developing targeted epigenetic therapies to mitigate the global burden.

PLAIN LANGUAGE SUMMARY

Heart disease, kidney disease, and metabolic conditions like type 2 diabetes and obesity are major health problems around the world. These diseases often occur together and are influenced by similar factors, such as genes, environment, and lifestyle choices. These factors could affect our health through epigenetics, which regulate the expression of genes without changing the DNA sequence. DNA methylation is an important type of epigenetic modifications, and can be accurately measured using high-throughput technologies. Population studies have found that certain DNA methylation patterns are linked to these diseases. These patterns can help us understand how these diseases start and progress. These DNA methylation markers may be used to improve early detection and prevention. In this review, we summarized recent research findings on DNA methylation and its connection to these chronic diseases. We found that some methylation patterns are shared across diseases, while others are more specific. New tools that combine multiple methylation markers into risk scores are promising for capturing cumulative disease risk, and improving disease prediction. More research with large, diverse study groups and advanced methods is needed to discover new markers and develop treatments that can target these changes.

ARTICLE HISTORY
Received 31 March 2025
Accepted 21 July 2025

KEYWORDS
Cardiovascular disease; coronary heart disease; heart failure; EWAS; DNA methylation; methylation risk score; longitudinal; incidence

1. Introduction

Cardio-kidney-metabolic (CKM) diseases pose a major risk to public health, affecting over 25% of U.S. adults and ranking as the leading cause of death [1–4]. The American Heart Association (AHA) has recently highlighted the interconnected nature of cardiovascular, kidney, and metabolic diseases, emphasizing their shared risks and prevention approaches [5]. Research continues to explore the shared mechanisms underlying CKM diseases, which include inflammation, metabolic dysfunction, and impaired adipose tissue, all playing key roles in disease progression. These complex pathobiological pathways contribute to the development and severity of CKM conditions. Given the high morbidity and mortality associated with CKM diseases, there is an urgent need for improving early detection and targeted interventions, which will ultimately help reduce the global burden. A more complete understanding of the disease risk profile is a key step toward improving CKM disease outcomes.

Complex mechanisms and pathophysiologies have been identified as underlying CKM diseases. Both genetic and environmental factors influence CKM disease risk. Although large genome-wide association studies (GWAS) have identified

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Article highlights

Epigenomic Studies of CKM Diseases
- Epigenetic modifications, particularly DNA methylation (DNAm), serve as crucial molecular mediators that connect genetic predisposition, environmental exposures, and lifestyle factors to disease development.
- Epigenome-wide association studies (EWAS) findings demonstrate that DNAm patterns dynamically change over time, playing a significant role in CKM disease progression and aging processes.
- Methylation risk scores that integrate multiple DNAm markers show promising potential for enhancing CKM disease prediction and risk assessment capabilities.

Clinical and Translational Implications
- DNAm biomarkers could enable early detection of CKM diseases, particularly in high-risk populations.
- Lifestyle interventions may modulate DNAm patterns, offering preventive strategies.
- Future research should integrate multi-omics data to clarify causal pathways and develop targeted epigenetic therapies.

Challenges and Future Directions
- Limited diversity in EWAS populations restricts the generalizability of findings; large, harmonized, and inclusive studies are needed.
- Advanced statistical methods and longitudinal designs are essential to unravel temporal DNAm changes and their clinical relevance.
- Translational efforts are needed to bridge the gap between epigenetic discoveries and clinical applications, such as personalized risk prediction and therapeutic interventions.

thousands of genetic loci associations with CKM disease traits [6–15], only a small proportion of the trait variance can be explained by these genetic loci jointly, limited by the overall genetic contribution to CKM disease risk, as well as potential gene-environment interaction effects. Since CKM disease typically has a later-in-life onset, time-varying risk factors are under-investigated, partly because accurately measuring these factors across the lifespan is not feasible at the population level. Human molecular markers, such as epigenetic factors, can be modified by environmental exposures, genetic factors, and physiological conditions, and thus, can serve as proxy biomarkers for CKM risk.

Epigenetics refers to molecular modifications that are unrelated to the primary DNA sequence and that can arise from environmental exposures [16]. Epigenetic modification, such as DNA methylation (DNAm), can regulate gene expression levels, and therefore, influence susceptibility to disease development [17]. Furthermore, epigenetic markers are modified by age [18,19], chronic inflammation [20,21], and environmental risk factors, such as exercise, smoking [22], and poor nutrition [23]. Thus, the epigenetic profile can capture many of the cumulative environmental and pathophysiological effects that influence susceptibility to CKM diseases. Recent advances in DNAm profiling technologies have significantly enhanced our ability to interrogate the methylome at high resolution and scale. While array-based platforms such as the Illumina Infinium HumanMethylation450K and EPIC arrays have been widely used due to their affordability and robust performance, they only cover preselected CpG sites and offer incomplete coverage of the methylome [24]. Other methods such as targeted bisulfite sequencing, whole-genome bisulfite sequencing (WGBS), and reduced representation bisulfite sequencing (RRBS) provide greater or complementary coverage, enabling a comprehensive assessment of methylation beyond the regions represented by the array-based platforms [24].

An epigenome-wide association study (EWAS) is a study of epigenetic markers, mostly DNAm, across the entire genome associated with a trait or disease of interest [19]. EWAS provides an unbiased approach for identifying molecular mediators of genetic and environmental factors that may explain the residual risk of disease [19]. Figure 1 illustrates how lifestyle factors dynamically induce DNAm changes that may lead to phenotypic variations across CKM systems, contributing to organ dysfunction through both shared and tissue-specific mechanisms. Longitudinal studies of epigenetics are crucial for understanding how molecular markers (e.g., gene expression) are influenced by environmental and lifestyle factors, as well as how those factors influence gene expression over time. Unlike static germ-line genetic profiles over the lifespan, epigenetic profiles track molecular changes (e.g., DNAm, histone modifications, and non-coding RNAs) across different life

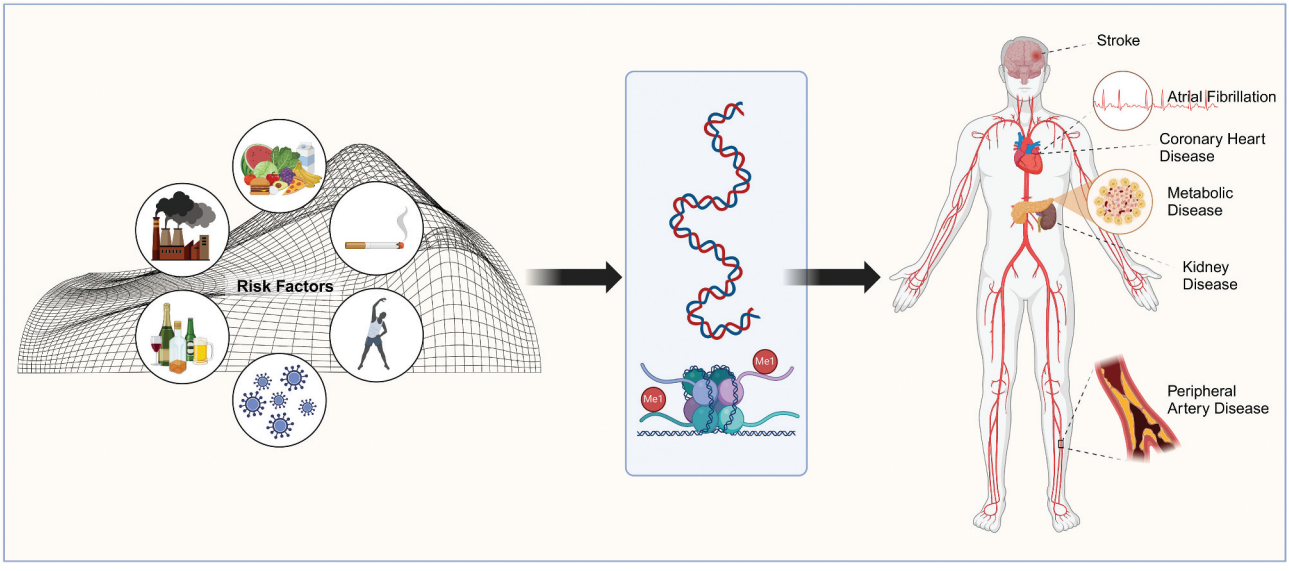

Figure 1. Epigenetic mediation of time-varying risk factors on cardio-kidney-metabolic diseases. Created in BioRender. Sun, Y. (2025) https://BioRender.com/bpubixs.

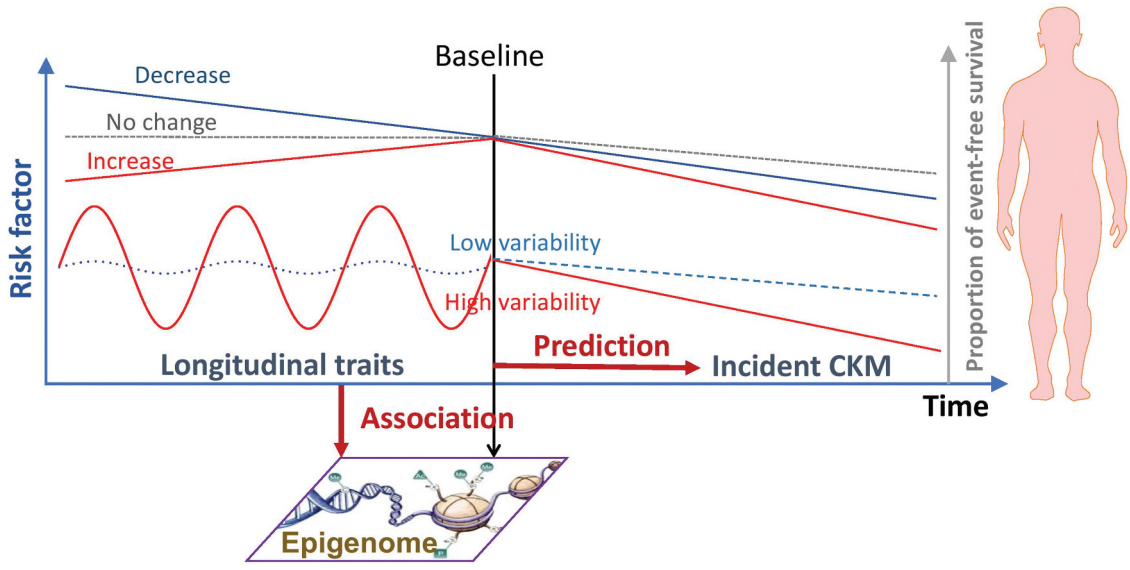

Figure 2. Conceptual model for epigenetic association studies of longitudinal phenotypes.

stages, providing insight into CKM disease development and aging processes. The longitudinal studies help identify biomarkers for early disease detection, predict long-term health outcomes, and assess the effectiveness of interventions. By analyzing epigenetic modifications over time, and epigenetic markers of longitudinal exposures, researchers can distinguish between transient and stable changes, improving causal inferences and risk predictions. Additionally, longitudinal approaches enhance personalized medicine by tailoring treatments based on an individual's epigenetic profile. Figure 2 presents a conceptual model for epigenetic association studies of longitudinal phenotypes, depicting how temporal measures of risk factors, DNAm, and phenotype changes (*e.g.*, trajectory and variability) may predict disease outcomes.

For this review, relevant articles were identified through targeted searches in PubMed using key terms related to Cardiovascular-Kidney-Metabolic (CKM) syndrome, coronary heart disease, heart failure, type 2 diabetes, obesity, kidney disease, DNAm, and EWAS. The search focused on literature published from 2014 to the present. We included English-language peer-reviewed articles and excluded non-peer-reviewed materials and studies outside the thematic scope of the review. Furthermore, we prioritized population-based studies with relatively large sample sizes, use of high-throughput DNAm data, and investigation of prevalent or incident disease cases. Selected DNAm studies of CKM diseases are summarized in Tables 1–3 and visually synthesized in Figure 3.

2. Coronary heart disease

Coronary heart disease (CHD) is the most common cardiovascular disease and the leading cause of death in the United States. An estimated 20.5 million Americans aged 20 and older have CHD, with an overall prevalence of 7.1% [64]. While the pathogenesis of CHD and its association with atherosclerosis is well established, the molecular mechanism of the disease's development and progression is complex [65].

Recent studies have increasingly recognized DNAm alterations as important contributors to the development of CHD [66–68]. Methylation levels of the repetitive long interspersed nuclear element-1 (LINE-1) are commonly used as a proxy for global DNAm, and its association with CHD has been established [30,69]. More gene-specific studies have further elucidated the mechanisms underlying CHD. For example, *APOE*, *APOC3*, *APOA5*, *LIPC*, *CETP*, and *APOC1* are critical for lipid metabolism and atherosclerotic development, influencing plaque formation in arteries. Studies have shown that altered DNAm of these genes is associated with atherosclerosis risk in CHD [70–72]. *ABCA1* is a crucial gene that promotes the transfer of cholesterol and phospholipids from cells to apolipoprotein A1, the major protein component of high-density lipoprotein. *ABCA1* methylation has been linked to altered lipid profiles and the development of CHD [73–76]. Methylation of *IL6*, which plays a central role in chronic inflammation, is a key factor in the development of atherosclerosis [77,78]. Further, A large-scale EWAS across nine population-based cohorts from the US and Europe found that DNAm at 52 CpG loci in blood leukocytes was associated with incident CHD, with several loci linked to genes involved in calcium regulation and kidney function [28]. Mendelian randomization (MR) analyses suggested a causal relationship between DNA methylation and CHD, and provided strong evidence that blood DNAm patterns are prospectively associated with incident CHD [28]. A study of CHD within a Chinese biobank reported 25 CpG loci including *SNX30*, which plays a crucial role in endocytic trafficking, homeostatic response, and cardiovascular disease [27]. Another study among a diverse population including American Indians [25] identified several methylation sites mapped to genes associated with coronary artery lesions and lipids, including *COL11A2* [79] and *FRMD5* [80,81]. A study of myocardial infarction, a common consequence of CHD, reported that differential DNAm of the *ZBTB12* gene plays a role in MI through potential cardiovascular signaling interactions [30]. Further, a study of acute myocardial infarction and incident CHD identified *AHRR*, *PTCD2*, and *MPO*, which are genes involved in key biological pathways such as inflammation, oxidative stress, and

Table 1. Selected DNA methylation studies of coronary heart disease and heart failure.

First author	Reference	Year	Disease	Sample size	Tissue type	Epityping platform	Population
Coronary Heart Disease							
Ana Navas-Acien	[25]	2021	Coronary Heart Disease	Discovery: 2,321 Validation: 1,874 + 2,128 + 2,114 + 931	Blood	Discovery: Illumina Infinium MethylationEPIC BeadChip Validation: Illumina Infinium HumanMethylation450 BeadChip	Discovery: American Indian Validation: African, Hispanic, European
Alba Fernández-Sanlés	[26]	2021	Acute Myocardial Infarction	Discovery: 196 cases, 195 controls Validation: 101 cases, 103 controls Follow-up analysis: 1,890 + 2,568	Blood	Discovery, Validation: Illumina Infinium MethylationEPIC BeadChip Follow-up analysis: Illumina Infinium HumanMethylation450 BeadChip	European
Jiahui Si	[27]	2021	Coronary Heart Disease	491 cases, 491 controls	Blood	Illumina Infinium MethylationEPIC BeadChip	East Asian
Golareh Agha	[28]	2019	Myocardial Infarction, Coronary Heart Disease	11,461	Blood	Illumina Infinium HumanMethylation450 BeadChip	European, African
Mathias Rask-Andersen	[29]	2016	Myocardial Infarction	48 cases, 681 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	European
Simonetta Guarrera	[30]	2015	Myocardial Infarction	Discovery: 292 cases, 292 controls Validation: 317 cases, 262 controls	Blood	Discovery: Illumina Infinium HumanMethylation450 BeadChip Validation: MALDI-TOF mass spectrometry methylation assay	Discovery: European Validation: European
Priyanka Sharma	[31]	2014	Coronary Artery Disease	Discovery: 18 cases, 18 controls Validation: 48 cases, 48 controls	Blood	Discovery: 12K Human CpG Island Microarray Validation: Bisulfite Sequencing	South Asian
Heart Failure							
Mark E Pepin	[32]	2019	Ischemic Heart Failure	Ischemic HF: 5 Non-ischemic HF: 6	Cardiac Tissue in the Apex of the Left Ventricle	Illumina Infinium HumanMethylation450 BeadChip	European, African
Nadezhda Glezeva	[33]	2019	Heart Failure (Ischemic Cardiomyopathy, Dilated Cardiomyopathy, Hypertrophic Obstructive Cardiomyopathy)	Cases: 9 Ischemic Cardiomyopathy, 9 Dilated Cardiomyopathy, 12 Hypertrophic Obstructive Cardiomyopathy Controls: 9	Cardiac Interventricular Septal Tissue	Bisulfite Sequencing	Unspecified
Benjamin Meder	[34]	2017	Heart Failure (Caused by Dilated Cardiomyopathy)	Discovery: 41 cases, 31 controls Validation 1: 18 cases, 8 controls Validation 2: 9 cases, 28 controls Validation 3: 82 cases, 109 controls	Left Ventricular Biopsies, Blood	Illumina Infinium HumanMethylation450 BeadChip	European

mitochondrial function, all of which contribute to the development of CHD [26].

3. Heart failure

Heart failure (HF) is a critical syndrome and clinical endpoint of multiple cardiovascular diseases. It is affecting approximately 7 million Americans, a number projected to increase to 8.5 million by 2030. HF is associated with high mortality rates and expensive healthcare costs [64,82,83]. Changes to the epigenome have been linked to HF and its hallmark features, such as cardiac hypertrophy and fibrosis [34,84–87].

A high-resolution EWAS revealed significant DNAm patterns in myocardial tissue of HF, linked to altered gene expression in cardiac development-related genes like *HAND2*, *PLXNA2*, *NPPA*, and *NPPB*, which are involved in cardiomyocyte differentiation, proliferation, and the production of atrial natriuretic factor and BNP, key biomarkers of cardiac stress and function [34]. Epigenetic modification has been observed in HF subtypes classified by different etiologies, including ischemic HF, a common endpoint of CHD. A study of patients undergoing coronary artery

Table 2. Selected DNA methylation studies of type 2 diabetes and obesity.

First author	Reference	Year	Disease	Sample size	Tissue type	Epityping platform	Population
Type 2 Diabetes							
Yinxia Su	[35]	2025	Prevalent Type 2 Diabetes	320 cases, 332 controls	Blood	QIAGEN Pyro Mark Q96 ID	East Asian
Robert F Hillary	[36]	2023	Incident and Prevalent Type 2 Diabetes	Incident: 534 cases 13,437 controls Prevalent: 348 cases 14,002 controls	Blood	Illumina Infinium MethylationEPIC BeadChip	European
Eliza Fraszczyk	[37]	2022	Incident Type 2 Diabetes	Discovery: 1,250 cases, 1,950 controls Validation: 1,072 cases, 1,587 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: European Validation: South Asian
Diana L. Juvinao-Quintero	[38]	2021	Prevalent Type 2 Diabetes	340 cases, 3,088 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	European
Karlijn A C. Meeks	[39]	2019	Prevalent Type 2 Diabetes	256 cases, 457 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	African
Alexia Cardona	[40]	2019	Incident Type 2 Diabetes	Discovery: 563 cases, 701 controls Validation 1: 1,074 cases, 1,590 controls Validation 2: 403 cases, 2,204 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: European Validation 1: South Asian Validation 2: European
Carolina Soriano-Tarraga	[41]	2016	Prevalent Type 2 Diabetes	Discovery: 151 cases, 204 controls Validation 1: 59 cases, 108 controls Validation 2: 63 cases, 582 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	European
Ines Florath	[42]	2016	Prevalent Type 2 Diabetes	Discovery: 153 cases, 835 controls Validation: 87 cases, 440 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	European
John C Chambers	[43]	2015	Incident Type 2 Diabetes	Discovery: 1,608 cases 11,927 controls Validation: 306 cases, 6,760 controls	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: South Asian Validation: European
Obesity							
Wenran Li	[44]	2024	BMI change	Discovery: 407 Validation: 126	Blood	Illumina Infinium MethylationEPIC BeadChip	Discovery: East Asian Validation: East Asian
Jacquelyn Y Taylor	[45]	2023	BMI	Discovery: 239 Validation: 961	Blood	Illumina Infinium MethylationEPIC BeadChip	Discovery: African Validation: African
Whitney L Do	[46]	2023	BMI	Discovery: 17034 Validation: 4,822	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: European, African, South Asian Validation: European, African, Hispanic, Asian, American Indian
Anne E Justice	[47]	2020	Waist Circumference, Waist-To-Hip Ratio, Waist-To-Height Ratio	Discovery: 2,684 Validation 1: 5,743 Validation 2: 820	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: African Validation 1: European, African, Hispanic Validation 2: European
Katherine C Crocker	[48]	2020	BMI, Waist Circumference, Body Fat Percentage	2,325	Blood	Illumina Infinium MethylationEPIC BeadChip	American Indian
Dianjianyi Sun	[49]	2019	BMI change	Phase 1 - Discovery: 1,485 Validation: 480 Phase 2 - 640	Blood	Illumina Infinium HumanMethylation450 BeadChip	Phase 1 - European, African Phase 2 - European, African
S Wahl	[50]	2017	BMI	Discovery: 5,387 Validation: 4,874	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: European, South Asian Validation: European, South Asian

BMI: Body mass index.

Table 3. Selected DNA methylation studies of kidney disease.

First author	Reference	Year	Disease	Sample size	Tissue type	Epityping platform	Population
Xueting Qi	[51]	2025	Estimated Glomerular Filtration Rate (eGFR)	244 pairs of monozygotic twins	Blood	Reduced Representation Bisulfite Sequencing	East Asian
Anna Syreeni	[52]	2024	Diabetic Kidney Disease Progression	Early Progression: 403 Late Progression: 373	Blood	Illumina Infinium MethylationEPIC BeadChip	European
Rachel KY Hung	[53]	2024	Chronic Kidney Disease (CKD), eGFR	Discovery: 27 cases, 55 controls with persistently normal kidney function, 37 with previous acute kidney injury or mild CKD. Validation 1: 2,376 cases 23,233 controls Validation 2: 4,667 Validation 3: 96 cases, 794 controls	Blood	Illumina Infinium MethylationEPIC BeadChip	Discovery: African Validation 1: African, European, Hispanic, South Asian Validation 2: African Validation 3: African
Charles E Breeze	[54]	2024	*APOL1* Risk Genotypes	Discovery: 410 Validation: 201	Blood	Illumina Infinium HumanMethylation450 BeadChip	African
Junyu Chen	[55]	2023	eGFR	Discovery: 885 with HIV Validation: 2,878 without HIV	Blood	Illumina Infinium HumanMethylation450 BeadChip Illumina Infinium MethylationEPIC BeadChip	African
Kelly Yichen Li	[56]	2023	eGFR, eGFR change	Discovery: 1,268 Validation: 326	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: East Asian Validation: American Indian
Robert F Hillary	[36]	2023	Incident CKD, Prevalent CKD	18,413	Blood	Illumina Infinium MethylationEPIC BeadChip	European
Laura J Smyth	[57]	2022	Prevalent Diabetic Kidney Disease	651 cases, 653 controls	Blood	Illumina Infinium MethylationEPIC BeadChip	European
Pascal Schlosser	[58]	2021	eGFR, Urinary Albumin-to-creatinine Ratio (UACR)	eGFR – Discovery: 22347 Validation: 11258 UACR – Discovery: 11458 Validation: 3,610	Blood	Illumina Infinium MethylationEPIC BeadChip Illumina Infinium HumanMethylation450 BeadChip Illumina Infinium HumanMethylation27 BeadChip	European
Charles E Breeze	[59]	2021	eGFR	Discovery: 5,428 Validation: 8,109	Blood	Illumina Infinium HumanMethylation450 BeadChip Illumina Infinium MethylationEPIC BeadChip	Discovery: African, European, Hispanic Validation: European, African
Farah Ammous	[60]	2021	eGFR	Discovery: 961 Validation: 614	Blood	Discovery: Illumina Infinium MethylationEPIC BeadChip Validation: Illumina Infinium HumanMethylation450 BeadChip	African
Xin Sheng	[61]	2020	Albuminuria, eGFR, eGFR change	473	Blood	Illumina Infinium MethylationEPIC BeadChip	African, European, Hispanic
Chengxiang Qiu	[62]	2019	eGFR change	Discovery: 181 Validation: 40	Blood	Illumina Infinium HumanMethylation450 BeadChip	Discovery: American Indian
Audrey Y Chu	[63]	2017	eGFR, CKD	2,264 + 2,595	Blood	Illumina Infinium HumanMethylation450 BeadChip	European, African

CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; UACR: Albumin-to-creatinine ratio.

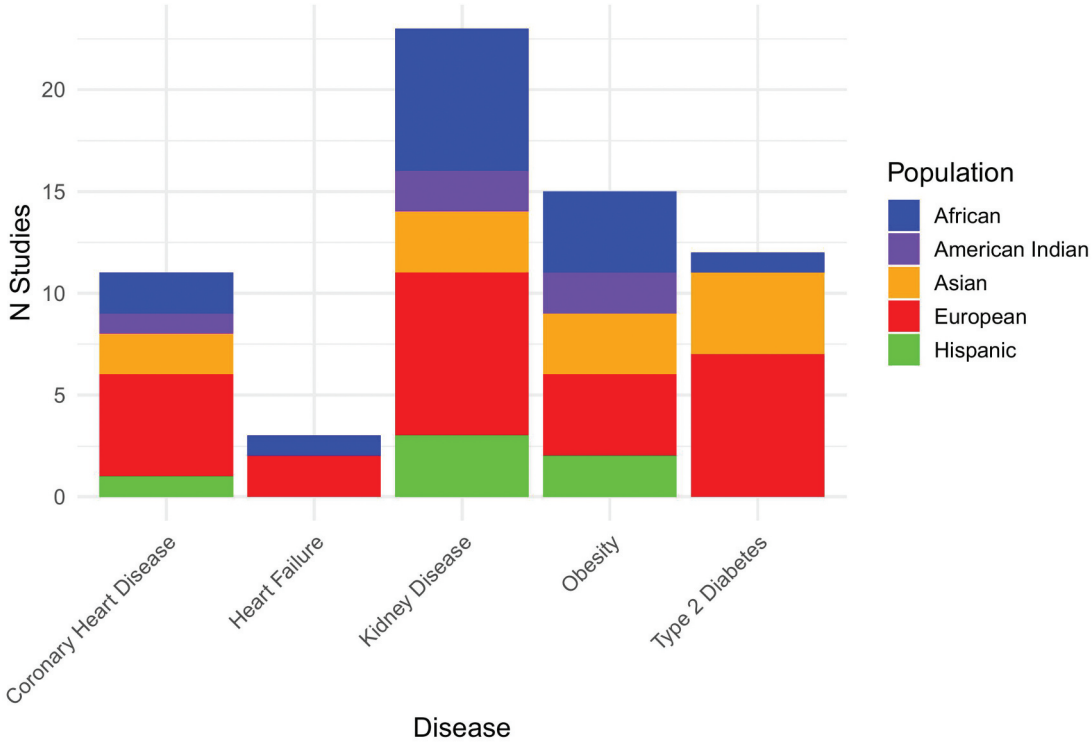

Figure 3. Histogram of the selected DNA methylation studies of CKM by disease domain and populations. Studies involving multiple populations are counted separately for each population included.

bypass graft surgery investigated DNAm changes associated with HF, revealing that *HDAC9*, *JARID2*, *GREM1*, and *PDSS2* play important roles in the pathogenesis of HF through mechanisms related to cardiac remodeling, fibrosis, and mitochondrial dysfunction [88]. Another study investigated human ischemic left ventricular tissue obtained from patients with end-stage HF and identified that DNAm plays a critical role in ischemic HF by reprogramming metabolic pathways through the epigenetic regulation of genes such as *KLF15* [32]. The findings suggest that epigenetic modifications mediated by *EZH2* are central to the transcriptional reprogramming observed in ischemic HF [32]. Classified by the underlying pathophysiology and functional impairments of HF, another study reported that the methylation of *COX17*, *MYOM3*, and *CTGF* plays a role in mitochondrial function and muscle contractility in ischemic cardiomyopathy; *CTGF* and *MMP2* may influence ventricular dilation in dilated cardiomyopathy; and altered methylation of *HEY2* and *MSR1* may drive heart development, inflammatory response and lipid homeostasis in hypertrophic obstructive cardiomyopathy [33]. However, despite these associations, there is currently no evidence demonstrating a causal relationship between DNA methylation changes and the development or progression of HF.

4. Diabetes

Diabetes mellitus is a complex metabolic disorder and remains a significant global health challenge. It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, which can manifest in two primary forms: Type 1 and Type 2. Type 1 diabetes is an autoimmune disorder where the body's immune system attacks and destroys insulin-producing beta cells in the pancreas. Type 2 diabetes (T2D), the more globally prevalent form, is often associated with insulin resistance and impaired insulin secretion [89,90]. Among individuals with T2D, cardiovascular diseases are the most common cause of death. This has become a global concern, given the rise in the prevalence of T2D in low- and middle-income countries [91].

An early cross-sectional study conducted in a Spanish discovery cohort in 2016 identified a locus within the *TXNIP* gene as being significantly hypomethylated in patients with T2D, and a subsequent in-silico analysis demonstrated that the binding site where the CpG site is located is associated with glucose import. These findings were replicated in two additional Spanish cohorts [41]. Between two subsamples of the ESTHER study in Germany, researchers found 39 CpG sites significantly associated with prevalent diabetes, including one site located in the *TXNIP* gene region [42]. The T2D-associated CpG site was also associated with inflammatory biomarkers independent of diabetes [20]. In addition to its known function within pancreatic beta cell biology, *TXNIP* gene plays a role as a physiologic regulator of peripheral glucose uptake through hyperglycemia and by uptake inhibition into fat and muscle [41,42]. More recent cross-sectional studies in European cohorts have uncovered novel associations at or near the *HDAC4*, *SYNM*, *MIR23A*, *ABCG1*, and *CPT1A* genes and confirmed the previously identified association within *TXNIP* [38]. Within the first study conducted in a cohort of sub-Saharan Africans, major findings included associations for loci at or near the *C7orf50*, *TPM4*, *VPS52*, *CDC42EP2* genes, and associations for loci within *CPT1A* and *TXNIP* were validated [39].

A large meta-analysis across five discovery EWASs in European populations and a validation within a population of Indian Asians living in England produced 76 CpG sites significantly associated with T2D. The strongest association was found to be with a locus within *TXNIP*, and other top associations were found within *ABCG1*, *CPT1A*, and *SREBF1*. [37] In addition to its similarities with the cross-sectional study approaches, these findings parallel those from prospective cohort studies conducted within sub-populations of two of the five discovery cohorts included in the meta-analysis (EPIC-Norfolk Study and LOLIPOP) [40,43]. By combining all of these studies' cohort data with information from 24,000 individuals from the Generation Scotland cohort, researchers further validated these previously observed associations, most notably those with genes *ABCG1*, *CPT1A*, *SREBF1*, and *TXNIP*. Importantly, there were also 58 novel associations with self-reported diseases including breast cancer, ischemic heart disease, and T2D [36]. Under a cross-lagged study design, associations were identified from baseline glycemic measures to subsequent DNA methylation, but not in the reverse direction, suggesting that DNAm may be consequences rather than causes of glycemic variation [92].

5. Obesity

Obesity, defined as a body mass index (BMI) over 30 kg/m^2, is a critical risk factor for cardiovascular diseases. According to the Global Burden of Disease, high BMI accounts for 4 million deaths annually, primarily due to coronary artery disease, HF, and other circulatory disorders [93]. Obesity is a multifactorial trait shaped by genetic predispositions and environmental exposures, which together significantly increase the risk of CKM diseases [10,94,95]. DNAm, influenced by environmental exposures, is crucial in modulating obesity risk by altering gene expression [19].

Studies have identified several CpG loci associated with BMI and related traits such as waist circumference (WC) and waist-to-hip ratio (WHR) [44,45,96–98]. A study identified a CpG locus associated with WC adjusted for BMI, emphasizing the importance of precise phenotype definitions in detecting specific associations [99]. Longitudinal studies and MR analyses have further suggested that BMI-associated DNAm changes predict obesity-related outcomes, such as coronary artery disease and T2D [50,100], strengthening their role as potential biomarkers for disease risk. It was found that obesity precedes and leads to changes in DNA methylation over time, suggesting that obesity is a cause rather than a consequence of these epigenetic alterations [49].

EWAS findings have implicated key genes in obesity-related pathways, shedding light on the molecular mechanisms underlying obesity. *SOCS3*, a gene that inhibits inflammatory cytokines and hormones, such as insulin and leptin, is critical in energy metabolism [101]. A meta-analysis of 13 EWASs comprising 22,310 participants of diverse ancestries identified *SOCS3* as part of enriched pathways and differentially expressed in adipose tissues [102]. MR studies have shown that *SOCS3* methylation levels are inversely associated with abdominal obesity (defined by WC), highlighting its role in obesity-related inflammation [103]. *ABCG1*, a gene critical for cholesterol efflux to high-density lipoprotein (HDL) and the prevention of cellular lipid accumulation, has been linked to cardiometabolic traits, including T2D, through its methylation levels [104,105]. Similarly, *SREBF1*, which regulates cholesterol synthesis and uptake, has been implicated in lipid metabolism pathways that contribute to obesity-related conditions [106]. *CPT1A*, encoding carnitine palmitoyltransferase 1A, facilitates the transport of long-chain fatty acids into mitochondria for beta-oxidation [107]. Methylation at *CPT1A* has been associated with visceral fat accumulation, metabolic dysregulation, and cardiometabolic diseases [108,109]. A study integrated metabolomics and epigenomics, and suggested that metabolite-driven causal effects on methylation at *CPT1A* are associated with obesity [110]. These findings provide functional insights into regulatory response mechanisms linked to obesity. Studies involving African-American and multi-ethnic Asian cohorts highlight the importance of diverse ancestries in EWAS, revealing both ancestry-specific and cross-population epigenetic signals associated with obesity [47,98]. A recent meta-analysis of 17,034 individuals from multi-ancestry cohorts uncovered 700 novel CpG loci, including five loci with interactions between BMI and race/ethnicity among individuals of European and African ancestry [46].

6. Kidney disease

Chronic kidney disease (CKD), defined as graduate loss of kidney function or abnormalities of kidney structure, affects over 10% of the general population across the world [111] and is closely linked with cardiovascular diseases [112]. Patients in early CKD stages exhibit an elevated risk of cardiovascular events even when vascular disorder clinical symptoms are absent [113], while cardiovascular-associated mortality constitutes approximately 40% to 50% of all deaths in patients with advanced CKD stages [114]. Several studies have explored DNAm alterations in association with diverse kidney-related phenotypes in hope of better understanding the pathologies of CKD.

In practice, the kidney function is most commonly assessed by the estimated glomerular filtration rate (eGFR) calculated from creatinine or cystatin C or both with information on age, biological sex [115]. The largest multi-ancestry blood-based EWAS meta-analysis of eGFR in the general population to date detected 69 eGFR-associated CpG sites [58]. The most significant CpG site in this study is near *ZNF788/ZNF20*, whose association with eGFR has been observed consistently across different ancestry groups [36,55,59,60,62]. However, its biological function remains unclear. Other top CpG sites include *JAZF1*, *ZSCAN29*, and *CDCP2*, which were shown to differentially methylated in kidney transplant patients pretransplant and post-transplant [116]. Another multi-ancestry meta-analysis of eGFR discovered 93 CpG sites associated with eGFR and 13 were successfully replicated [59]. One of the identified CpG sites was located within an intron of *HSP90AA1*, which is involved in protecting kidney tissue from inflammation, ischemia, and oxidative damage, assisting with cellular repair [117]. Its encoded protein HSP90 regulates eGFR through the nitric oxide pathway and is a drug target candidate for kidney

diseases [117]. Both of these meta-analyses conducted ancestry-specific analysis of European ancestry and African ancestry, and reported that there were little overlap between ancestry-specific findings [58,59]. Larger samples inclusive of multiple ethnicities are needed to further define ancestry-specific DNAm signatures for eGFR.

The human immunodeficiency virus (HIV) infection can lead to progressive decline in renal function, known as HIV-associated nephropathy (HIVAN) [118]. It was suggested that HIV infection could modify the associations between DNAm and kidney function [55], thus EWAS findings from the general population might not be fully extrapolated to HIVAN. Moreover, African descendants have a markedly higher risk of developing kidney diseases including HIVAN compared to other ancestry groups [119]. Thus, an EWAS of eGFR was conducted among people with HIV of African ancestry, in which 3 hypermethylated CpG sites were found to be associated with reduced eGFR, including a previously unidentified CpG site in *SHANK1* [55]. Furthermore, the high burden of kidney diseases including HIVAN among people of African ancestry has partially been attributed to two genetic variants (G1 and G2) of the apolipoprotein L1 (*APOL1*) gene [120]. People with homozygosity or compound heterozygosity for the G1 and G2 alleles (G1/G1, G2/G2 or G1/G2), termed *APOL1* high risk genotype, are at a higher risk of HIVAN. One recent EWAS aimed to investigate DNAm patterns associated with eGFR and CKD among people of African ancestry with HIV and *APOL1* high-risk genotype [53]. This study reported several novel eGFR/CKD-associated CpG sites located in *SCARB1*, *DNAJC5B* and *C4orf50*. To better understand the role of DNAm in kidney diseases attributed to *APOL1* high-risk genotype, alternatively, another study identified 5 differentially methylated CpGs that are proximal to and are influenced by *APOL1* risk variants in 611 African Americans [54]. These DNAm changes may help explain the kidney disease risk and clinical manifestation of *APOL1* high-risk genotype.

DNAm also plays an important role in the pathogenesis of many other forms of kidney diseases, such as diabetic kidney disease (DKD) [61,116], IgA nephropathy (IgAN) [121] and lupus nephritis [122]. Two EWAS of eGFR conducted among diabetic patients, respectively, in the US and Hong Kong revealed numerous differentially methylated CpG sites in DKD. While a large proportion of these findings overlap with EWAS of eGFR in the general population, there are novel DKD-associated CpG sites such as *RFTN1* and *CTSB* [61,116]. Both studies additionally investigated the association of DNAm with the rate of kidney function decline over time among diabetic patients and found significant CpG sites, which presented distinct DNAm signature from the general population. Smyth et al. performed an EWAS of DKD in patients with type-1 diabetes and identified 32 differentially methylated CpG sites, including *SLC27A3*, *FKBP5* and *TXNIP* [57].

Majority of the current EWAS for kidney-related phenotypes is that the DNAm are usually measured in peripheral blood, whose changes might be different in kidney tissues. A public dataset of Kidney-tissue-based DNAm in 506 microdissected kidney tissues samples have been used to replicate EWAS findings [58]. The similarity in findings between tissues indicates that changes observed in blood samples could be relevant for kidney tissue samples [58,61,62]. An EWAS of eGFR was conducted in 399 kidney tubule samples and identified 19 significant CpG sites [123]. The strongest signal was in the enhancer region of *UVSSA*. These findings are based on cross-sectional associations, and there is currently no direct evidence establishing that the identified methylation changes causally influence kidney function.

7. Lifestyle factors

Lifestyle plays a crucial role in the development of CKM diseases. Findings from large cohort studies have estimated that 90% of diabetes [124], 80% of CHD [125], 78% of HF [126], and 70% of CKD [127], could be attributable to unhealthy lifestyles. DNAm has been proposed to be one of the molecular mechanisms mediating the relationship between lifestyle factors and CKM diseases and population studies have been using blood derived DNAm to investigate such relationship.

An investigation in the Framingham Heart Study [128] demonstrated that a higher healthy life's essential behavior score built on sleep, physical activity, diet and smoking, was associated with a younger DNAm-based epigenetic age, independent of chronological age. Epigenetic age biomarkers partly mediate the associations between behavior score and incident cardiovascular diseases with an estimated mean proportion of mediation at 47% [128]. Utilizing genetic variants, a study reported that smoking-related DNAm markers in *REST*, *CLIP3*, *ARHGEF26*, *ARID5B*, and *MCF2L* may be causal to cardiovascular diseases [129]. An EWAS combining data in four cohorts from Spain, the USA, and the UK identified 18 differentially methylated CpGs associated with dietary scores. Of the 18 CpGs, 12 were associated with cardiometabolic traits [130]. Furthermore, the MR analysis of this study showed that DNAm levels associated with higher diet quality, e.g., hypermethylation for *PRMT1* and *AHRR*, were associated with lower risk of obesity, T2D, and CHD [130]. These findings were in line with another study [131] showing that DNAm mediated approximately 20% to 40% of the association between diet quality scores and all-cause mortality. Recent studies [132,133] also provided promising evidence supporting the potential mediating role of DNAm, e.g., methylation levels of *PIK3CD* and *VPS13D*, on physical activity's protective relationship with cardiovascular diseases. In summary, current literature provides strong evidence linking DNAm with both lifestyle and CKM risk, supporting differential DNAm represents a key underlying pathway leading to CKM diseases [134,135].

8. Longitudinal methylation studies and statistical methods

Early research in the field predominantly relied on cross-sectional designs. However, the shift toward longitudinal approaches has enabled a more nuanced understanding of how DNAm patterns evolve [136–140]. These approaches offer insights into aging processes [141–145], health indicators (e.g., BMI, blood pressure, glucose), disease progression [44,143,146–149], and responses to environmental stimuli

[150,151]. Most of these studies have up to three repeated measurements, and sample sizes range from hundreds to thousands. Normalized beta values have been widely used for these analyses.

Linear mixed-effects models (LMEs) and their extensions (e.g., hierarchical or multilevel models) are extensively employed in longitudinal methylation studies to characterize both individual-specific trajectories and overall population trends, Figure 2. By incorporating a random intercept, these models allow for individual baseline variations in methylation levels while including a random slope, which accounts for differential rates of change over time among subjects. By incorporating both random intercept and random slope, the more complex correlation structure is enforced between repeated measures within individuals. Most studies have only up to three repeated measurements, so they may only afford to incorporate random slopes in LME [143,152]. With > 3 measurements, studies can also enable nonlinear trend detection [152]. LME framework can readily accommodate time-varying covariates, such as measures of disease activity over time, as well as assessing the time trend between conditions (group by time interaction). As a result, LMEs provide an ideal methodological approach for identifying methylation sites that exhibit significant temporal patterns. Interindividual variability of DNAm is a mechanism of epigenetic drift in aging. Wang et al. then proposed an extension of LME to estimate interindividual methylation variability over time [145].

In certain cases, incorporating only random intercepts and slopes within an LME may be insufficient to capture the full complexity of the correlation structure in the data. This shortfall can be mitigated by introducing additional correlation structures in the residuals, such as autocorrelation models [147]. Alternatively, linear regression with cluster-robust standard errors, employing sandwich estimators to account for non-independence within clusters, can yield robust variance estimates even when the correlation structure is mis-specified [153]. This method can be faster than multilevel models while retaining comparable accuracy for identifying age- or exposure-related CpGs.

It is increasingly recognized that numerous risk factors, lifestyle choices, and environmental exposures influence disease etiology through epigenetic mechanisms, such as DNAm. Consequently, a variety of mediation analysis approaches have been developed to tackle the challenges posed by the high-dimensional nature of methylation data when these markers serve as mediators [154]. However, robust statistical methodologies that concurrently address both high-dimensional mediators and longitudinal study designs remain scarce. Only a limited number of studies have employed cross-lagged panel analysis to examine the dynamic interplay between exposures and DNAm, with measurements restricted to two discrete time points [49,92]. Cross-lagged panel analysis is a specialized form of path analysis. It adopts the structural equation modeling approach [155], and assesses reciprocal, longitudinal relationships among variables [156,157]. Thus, it offers a framework for understanding the bidirectional influences between exposures and methylation over time. Recently, a novel methodological framework has been introduced that extends traditional mediation analysis to accommodate the dual challenges of high-dimensionality and longitudinal data structures. This new approach was based on both an LME model and a generalized estimating equation. It employs sure independence screening to reduce the dimension of candidate mediators. Subsequently, the Sobel test with the Bonferroni correction for IE hypothesis testing can be implemented [158]. More methods that can handle different types of outcomes in longitudinal mediation analysis for methylation data are needed.

9. Methylation risk score

Methylation risk score (MRS) is a novel approach that combines individual methylation associations to jointly estimate the disease risk. MRS typically have larger effect sizes and can reduce the number of tests to improve statistical power in the study of CKM diseases. The MRS can be based on a weighted sum of identified DNAm sites associated and lifestyle factors (e.g., smoking, physical activity) and CKM risk factors (e.g., age, BMI) from published studies [46,159,160]. Using a training-testing approach, the MRS can be developed by optimizing the prediction of a trait of interest (e.g., R^2 for a quantitative trait, and area under curve [AUC] of a binary disease outcome). For example, MRS of CKM diseases have been linked to cardiovascular outcomes [161], CKD [162], and metabolic disorders such as T2D [163] and obesity [164]. Advanced methods for constructing MRS, such as penalized regression models (e.g., LASSO, ElasticNet, ridge regression, adaptive LASSO) and tree-based machine learning methods (e.g., decision trees, random forests, gradient boosting), can be applied to address high-dimensionality and complexity of EWAS data. These approaches leverage large datasets and more sophisticated algorithms to enhance the precision and generalizability of the scores across diverse populations. Additionally, integrating MRS with multi-omics data, such as proteomics and metabolomics, holds the potential to further refine disease prediction and provide insights into underlying biological mechanisms, improving risk stratification and therapeutic targeting in CKM diseases. In the training stage, cross-validation approach can be used to summarize and compare the prediction performance between different machines and identify the most important predicators across multiple models. Ideally, the MRS needs to be validated in independent samples, particularly cross geographic regions, population and demographic groups to demonstrate generalizability and transferability of potential utility.

10. Future perspective

Although CKM diseases include very complex genetic and environmental causes and present unique clinical profiles across individual disease systems, they share risk factors, underlying pathophysiologies, and molecular mechanisms [5]. Epigenomic studies have showed the capability of identifying robust DNAm markers associated with CKM diseases. DNAm sites from several genetic regions (e.g., *TXNIP*, *ABCG1*, *SOCS3*) were associated with several CKM diseases, supporting common epigenetic mechanisms across CKM diseases. While these

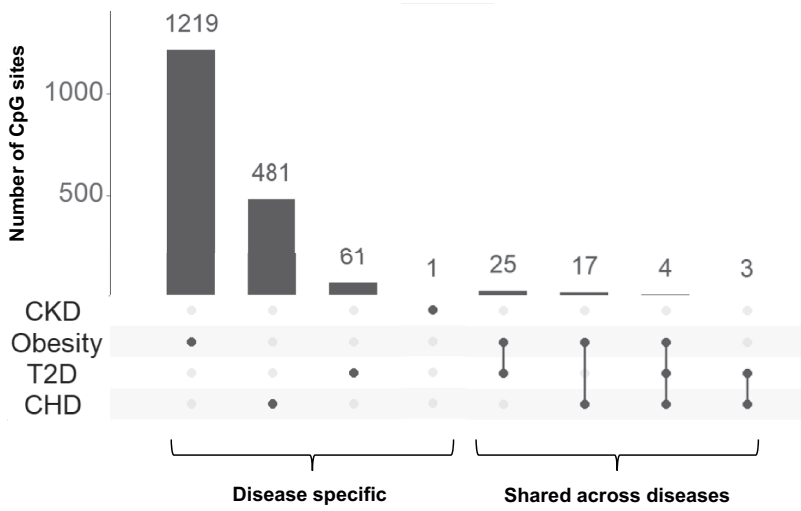

Figure 4. Shared and disease-specific CpG sites identified in large epigenome-wide association studies of coronary heart disease (CHD), type 2 diabetes (T2D), obesity, and chronic kidney disease (CKD) [25,36,46].

epigenetic markers are not disease-specific, their shared associations diverse exposures and traits suggest their core biological processes such as inflammation, oxidative stress, and metabolic dysregulation. This commonality across traits underscores their potential utility as integrative biomarkers of overall CKM disease risk, rather than indicators of a single disease pathway. Since EWAS of individual CKM diseases cannot maximize the power of identifying shared epigenetic associations across multiple disease traits, we sought to compare CKM diseases-associated DNAm markers using published EWAS findings [25,36,46], Figure 4. Substantial heterogeneity in sample sizes, tissue sources, analytical methods, and significance thresholds across studies limited the robust comparisons cross CKM diseases. The minimal overlap observed among reported CpG sites may reflect these methodological inconsistencies rather than unshared epigenetic markers across CKM diseases. These challenges underscore the need for harmonized, large-scale EWAS efforts across multiple CKM conditions, ideally leveraging consistent study designs and standardized analytic pipelines. Future work integrating multi-disease analyses within a unified framework may help disentangle shared versus disease-specific epigenetic signatures and provide deeper insight into common biological pathways underlying cardiometabolic and kidney disease risk. Recent efforts in large biobank studies such as the Million Veteran Program (MVP) and the All of Us programs will also offer opportunities of epigenetic research in CKM diseases, particularly with harmonized clinical outcomes and longitudinal phenotypes.

Limited diversity in EWAS presents a significant challenge for the generalizability and translatability of findings across populations. Most EWAS to date have been conducted in populations of European ancestry, limiting the applicability of DNAm markers to other racial and ethnic groups. This lack of diversity also hinders efforts to assess causality using methods such as MR, which require population-specific methylation quantitative trait loci (mQTLs) as genetic instruments. Expanding EWAS to include ancestrally diverse populations is essential to ensure equitable discovery, improve the robustness of causal inference, and identify epigenetic markers relevant across different genetic and environmental backgrounds.

Although drugs that directly target DNAm, such as DNA methyltransferase inhibitors, have been approved in oncology [165], their application in nonmalignant chronic diseases remains limited. Establishing causal relationships between DNA methylation and CKM diseases is a critical next step before DNAm markers can be considered as potential therapeutic targets or intervention. While many epigenetic associations have been identified, distinguishing causal effects from confounding or reverse causation remains a major challenge. Robust causal inference methods, such as two-step epigenetic MR [166] and longitudinal mediation analysis [158] are essential for evaluating the directionality and biological relevance. Translational research will progress from epigenetic biomarker discovery to clinical utility, with growing interest in integrating DNAm profiles into precision medicine approaches for early detection, prevention, and individualized therapeutic strategies in CKM conditions. Through independent and complementary effects with other -omic systems, epigenomic markers are valuable for early detection and disease prediction CKM diseases [167]. As technology advances, integrating multi-omics data with longitudinal epigenetic studies will further refine our understanding of gene-environment interactions and their joint impact on human health.

Author contributions

Chang Liu and Yan V. Sun led the drafting of the original manuscript. Alexandra Young, Nanzha Abi, Junyu Chen, Matheus Fernandes Gyorfy, Yanping Li, and Jin J. Zhou contributed to the drafting of the manuscript. Sophia Sun contributed to software and data visualization. Yan V. Sun provided overall supervision, conceptual guidance, methodology development, funding acquisition, and critical review and editing of the manuscript. All authors reviewed and edited the final version of the manuscript.

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes

employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
No writing assistance was utilized in the production of this manuscript.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Funding

This manuscript was partly funded by HL154996 and DK125187 from U.S. National Institutes of Health, BX005831 and BX006990 from U.S. Department of Veterans Affairs.

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