Clinical Epigenetics

REVIEW

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DNA methylation in cardiovascular disease and heart failure: novel prediction models?

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Abstract

Background Cardiovascular diseases (CVD) affect over half a billion people worldwide and are the leading cause of global deaths. In particular, due to population aging and worldwide spreading of risk factors, the prevalence of heart failure (HF) is also increasing. HF accounts for approximately 36% of all CVD-related deaths and stands as the foremost cause of hospitalization. Patients affected by CVD or HF experience a substantial decrease in health-related quality of life compared to healthy subjects or affected by other diffused chronic diseases.

Main body For both CVD and HF, prediction models have been developed, which utilize patient data, routine laboratory and further diagnostic tests. While some of these scores are currently used in clinical practice, there still is a need for innovative approaches to optimize CVD and HF prediction and to reduce the impact of these conditions on the global population. Epigenetic biomarkers, particularly DNA methylation (DNAm) changes, offer valuable insight for predicting risk, disease diagnosis and prognosis, and for monitoring treatment. The present work reviews current information relating DNAm, CVD and HF and discusses the use of DNAm in improving clinical risk prediction of CVD and HF as well as that of DNAm age as a proxy for cardiac aging.

Conclusion DNAm biomarkers offer a valuable contribution to improving the accuracy of CV risk models. Many CpG sites have been adopted to develop specific prediction scores for CVD and HF with similar or enhanced performance on the top of existing risk measures. In the near future, integrating data from DNA methylome and other sources and advancements in new machine learning algorithms will help develop more precise and personalized risk prediction methods for CVD and HF.

Keywords Cardiovascular disease (CVD), Heart failure (HF), DNA methylation, Prediction models

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Background

Cardiovascular diseases (CVD) affect over half a billion people globally and are a leading cause of global deaths, claiming about 17.9 million lives each year, approximately 30% of all global deaths, and imposing a heavy burden over healthcare systems [1]. CVD encompasses conditions affecting the heart, the blood vessels or both and include coronary artery (CAD), cerebrovascular, or peripheral artery diseases, and aortic atherosclerosis [1]. The heavy burden of CVD is attributed to a combination of socio-economic, metabolic, individual, behavioural, and environmental risk factors [1]. These include high blood pressure (BP), high cholesterol, diabetes, air pollution, obesity, chronic kidney diseases, aging, personal and familial CVD history, and unhealthy lifestyles, i.e. poorly diet, smoking, physical inactivity, harmful alcohol use, stress, etc. [1].

The prevalence of heart failure (HF) is increasing globally, due to a combined increase in the incidence and the improving survival of the disease. [2]. HF affects 56.2 million people worldwide, contributes to about 36% of all CVD deaths, and is the leading cause of hospitalization [2, 3]. HF is a clinical syndrome characterized by a reduced ability of the heart to pump or fill with blood, revealed by increased left ventricular (LV) filling pressure along with associated CV risk [4]. There are four stages of HF termed A, B, C, and D [5]. Stage A is a preclinical condition including individuals at high risk of developing HF due to related conditions such as hypertension, diabetes, and CAD. Stage B may include subclinical changes such as ventricular systolic or diastolic dysfunction, LV hypertrophy, chamber enlargement, valvular disease, and/or biochemical evidence of increased filling pressures, without symptoms of HF. In contrast, individuals at stages C and D have clinical evidence of HF. The initial diagnosis of HF is based on symptoms, i.e. fluid retention, exertional dyspnea, fatigue, and/or oedema, clinical findings, and test results with exclusion of other potential causes [5]. Diagnostic tests include laboratory testing, 12-lead electrocardiogram, and biomarker testing for natriuretic peptides (NT-proBNP and BNP) [5]. Echocardiography is crucial in establishing the diagnosis and in differentiating HF based on LV ejection fraction (LVEF) [5]. HF patients with an LVEF of 50% or more are classically defined as HF with preserved LVEF (HFpEF), those with a LVEF between 41 and 49% are considered to have mildly reduced EF (HFmrEF), and those with LVEF of 40% or less as having HF with reduced ejection fraction (HFrEF) [5]. Patients may occasionally fluctuate between different forms, making classification and pathogenetic interpretation more complex.

CVD and HF have significant effects on quality of life (QoL) and healthcare costs. In particular, a relevant fraction of healthcare expenditure is due to hospitalizations. Thus, reduction of the total costs for hospitalization caused by disease events is crucial for cost-effective prevention, diagnosis, and treatment strategies [4, 6]. Financially convenient interventions should focus on reducing the need for hospitalizations and re-hospitalizations, by the means of reducing CVD and HF incidence and improving disease progression and prognosis. Positive changes in lifestyles and environment as well as improved disease management have been linked to reduced incidence, morbidity, disease severity, and mortality for CVD [6]. As a consequence, these measures could also lead to a decrease in the impact of these diseases on QoL and healthcare costs. An effective strategy to minimize the overall burden of CVD and HF requires an impactful approach on the incidence and the prevalence of the diseases. Crucial to such strategies is the search of biomarkers capable to identify individuals at risk for CVD or HF, patients with faster progression, worse prognosis and higher risk of hospitalization and mortality. Research on CVD and HF on risk prediction by using innovative approaches is rapidly advancing [7, 8]. This expanding field has the potential to significantly enhance overall healthcare management, by assisting healthcare professionals in their clinical decision-making process and informing individuals on their risks of developing CVD or HF or more rapidly progressing towards an unfavourable outcome. In the following paragraphs, we will discuss currently available risk prediction tools for CVD and HF and novel strategies for advanced risk prediction.

Main text

Approaches to the prediction of CVD

Preventing CVD and its complications in clinical practice mainly focuses on identifying and addressing risk factors [9]. Identifying individuals at high risk of CVD or early diagnosis can reduce the overall burden of the disease and its mortality. To meet this need, various risk prediction models have been developed to assess individual CVD risk in adult patients and some of them may also provide information on current heart ageing [10–15]. Heart aging involves various cardiac tissues and can be associated with a higher risk of CVD [16]. CV risk calculators are now widely used and the number of available calculators on the web continues to grow [17]. Traditional risk prediction models, such as the Framingham Risk Score (FRS), are commonly used to calculate an individual's 10-year primary CVD risk in asymptomatic patients [10]. The FRS utilizes input variables including age, sex, total and high-density lipoprotein (HDL) cholesterol plasma levels, systolic BP and smoking status. However, the FRS has limitations such as reduced accuracy in some

ethnic groups and exclusion of certain risk factors [10]. International guidelines recommend the use of further risk prediction tools [18]. In the USA, the ASCVD (atherosclerotic CVD) score is a modern frequently adopted alternative to the FRS [11], while, in Europe, SCORE2 and SCORE-OP are commonly used to estimate CV risk [12, 13]. In the UK, QRISK3 is largely used because of the advantage of incorporating additional risk factors compared to FRS and of providing a more comprehensive assessment [14]. However, recent research has identified conditions associated with increased CVD risk that are not captured by the CVD risk equations most commonly adopted [15]. The new QR4 risk score, generated by using data from millions of adults in the UK and incorporating additional risk factors, such as brain, lung cancer, oral, and blood cancers, Down syndrome, COPD, and learning disability and, in women, pre-eclampsia and postnatal depression, shows superior performance compared to previously existing tools [15]. This increased accuracy and comprehensive risk assessment might improve patient outcomes through more personalized and effective preventive strategies. It is important to note that the same individual can receive different heart age or CV risk results depending on which calculator is used [16]. This discrepancy is influenced by the risk model adopted, the thresholds set for risk factors, and how the risk is translated into heart age or CV risk [16]. Furthermore, web-based calculators for heart age are inferential tests that do not directly consider heart physiology [19].

Given the complexity of heart and blood vessels diseases, imaging, electrocardiography (ECG) and machine learning approaches have been proposed for CVD risk assessment and heart aging [19–24]. Raisi-Estabragh et al. have developed an innovative model to estimate biological heart age using radiomic phenotypes of cardiac shape and myocardial features derived from CV magnetic resonance imaging [20]. These authors used data from 29,996 UK Biobank participants without CVD and extracted 254 radiomic information on the LV, right ventricle and the myocardium of each participant. Utilizing Bayesian ridge regression with tenfold cross-validation, they successfully built a model that accurately estimates heart age and heart age delta, i.e. the difference between the chronological age and model-estimated heart age. Additionally, the model shows strong correlations between heart aging and CV risk factors such as obesity, serum lipid markers, hypertension, diabetes and heart rate and represents a new method for phenotypic assessment related to CV aging [20]. In 2011, Starc et al. and, in 2014, Ball et al. developed statistical models to predict heart age using ECG data [19, 21]. Ball's method, which is based on a Bayesian approach, used a database of 5-min ECG recordings from healthy individuals and a validation group with cardiac risk factors and diagnosed cardiac disease [19]. The model aligned with the true chronological age in healthy individuals and showed higher predicted heart ages in subjects with risk factors and patients with proven heart diseases [19]. Since then, other ECG-based Heart Age approaches have been developed using Bayesian and artificial intelligence methods [22, 23]. Lindow et al. adopted a machine learning-based approach to predict cardiac age using information from a 10-s resting 12-lead ECG [22]. They analysed a database of ECG from 2,771 subjects, including healthy volunteers, individuals with CV risk factors and patients with CVD. The 10-s Heart Age showed strong agreement with the 5-min Heart Age. The Heart Age Gap from the true chronological age increased with CV risk and disease [22]. Ribeiro et al. developed a deep neural network (DNN) to estimate cardiac age based on raw 12-lead ECG tracings [23]. The DNN outperformed cardiology resident medical doctors in recognizing 6 types of abnormalities in ECG recordings, 1st-degree atrioventricular block, right bundle branch block, left bundle branch block, sinus bradycardia, atrial fibrillation and sinus tachycardia, with F1 scores above 80% and specificity over 99% [23]. Also, Hughes et al. recently developed a risk score called SEER (Stanford Estimator of Electrocardiogram Risk) using a deep convolutional neural network to accurately predict the long-term risk of CV mortality and disease based solely on a resting ECG [24]. SEER has shown impressive performance, accurately predicting 5-year CV mortality with an area under the receiver operator characteristic curve (ROC-AUC) of 0.83, 0.78, and 0.83 when evaluated on three independent populations. Additionally, SEER can predict 5-year ASCVD and other CV conditions such as HF and atrial fibrillation [24].

Approaches to the prediction of HF

Identifying individuals at stage A (high risk for HF) or those with stage B HF (without symptoms but with structural/functional cardiac abnormalities or elevated biomarkers) will enable earlier implementation of effective strategies to prevent or delay the progression to advanced HF [7]. However, HF stems from a variety of causes making it challenging for a single risk prediction model to encompass all at-risk individuals [7]. While traditional CV risk factors continue to be the primary contributors to the overall burden of HF in the population, incorporating nontraditional risk factors into HF risk assessment should enable to better accommodate the diversity within HF [7]. The HF risk models, based on FHS for 10-year risk [25] and the Health, Aging, and Body Composition (Health ABC) study for 5-year risk [26], incorporate traditional clinical risk factors such as age, systolic BP, heart rate, LV hypertrophy (LVH) and CAD. The first

model includes type 2 diabetes (T2D) and body mass index (BMI) [25], while the second model includes smoking and routine laboratory values [26]. However, in both models, CAD was found to be the strongest predictor of incident HF, restricting their use mainly to secondary prevention [25, 26]. The Pooled Cohort equations to Prevent HF (PCP-HF) addressed this limitation [27]. The 10-year risk model, developed in 5 diverse cohorts, excludes individuals with baseline ASCVD and demonstrates good discrimination (AUC ranging from 0.71 to 0.88) in all tested cohorts [27]. Furthermore, precise risk prediction models tailored for HFpEF and HFrEF have been developed [28]. The HFpEF-specific model included age, sex, systolic BP, BMI, antihypertensive treatment and previous myocardial infarction (MI), while the HFrEF-specific model additionally included smoking, LVH, left bundle branch block and T2D [28]. Furthermore, given the 22% prevalence of HF in individuals with diabetes, additional specific scores, such as the QDiabetes and the WATCH-DM, have been developed to predict HF risk in diabetes [29, 30].

In addition to HF prevention, understanding the prognosis of patients with HF is also needed [7]. Indeed, while some patients experience prolonged periods without hospitalization, others face an unstable clinical course with frequent HF decompensation and a poor prognosis [31]. The Seattle Heart Failure Model (SHFM) was the first web-based tool to predict survival for patients with HF both at baseline and after treatments [32]. This model was developed using data from 1125 patients and validated using data from 5 additional groups totalling 9942 patients. The SHFM takes into account clinical variables, medical treatment and standard laboratory tests to accurately estimate survival rates from 1 to 5 years. Additionally, it helps to estimate the potential benefit of adding medications or devices to patient's treatment plans [32]. There are other powerful risk scores currently used in clinical practice to predict mortality and morbidity in HF patients [33–35]. The Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC-HF) risk score is based on 13 independent predictors of mortality, including age, EF, serum creatinine, New York Heart Association (NYHA) class and diabetes. It predicts all-cause mortality at 1 and 3 years in patients with HF, whether they have reduced or preserved LVEF [33]. The PARADIGM Risk of Events and Death in the Contemporary Treatment of Heart Failure (PREDICT-HF) is based on standard clinical and laboratory data, including natriuretic peptides [34]. It accurately predicts morbidity and mortality in ambulatory patients with chronic HFrEF at 1 and 2 years [35]. The Barcelona Bio-Heart Failure (BCN-Bio-HF) risk calculator is based on clinical and routine laboratory data, with the addition of biomarkers like NT-proBNP, high-sensitive troponin T (hs-TnT) and high-sensitivity soluble ST2 [35]. This tool enables individual prediction of death at 1, 2 and 3 years in patients with at least one HF hospitalization or LVEF < 40% [35]. Recently, machine learning methods have been used to categorize different types of HF [36, 37]. In 2015, Shah et al. were among the first to conduct studies aimed at evaluating whether unbiased clustering analysis using dense phenotypic data could identify distinct categories of HFpEF [36]. By adopting advanced statistical learning algorithms and unbiased hierarchical cluster analysis, the authors developed a new sub-classification for HFpEF based on phenotypic data, including clinical, laboratory, ECG and echocardiographic data from 397 patients. This analysis identified three distinct groups of patients with significant variations in clinical characteristics, cardiac structure/function, invasive haemodynamics and outcomes. Notably, group 3 displayed higher risk of HF hospitalization even after adjusting for traditional risk factors [36]. Very recently, Banerjee et al. conducted a further investigation using machine learning methods to subtype and predict HF outcomes by analysing data from electronic health records [37]. The study included 313,062 patients from The Health Improvement Network and Clinical Practice Research Datalink databases, cross-referenced with the Hospital Episode Statistics, the UK death registry and the UK Biobank. Four unsupervised machine learning methods—K-means, hierarchical, K-Medoids and mixture model clustering—were used to identify subtypes, which were validated for external validity (across datasets), prognostic validity (predictive accuracy for 1-year mortality) and genetic validity (association with polygenic risk score for HF-related traits and single nucleotide polymorphisms). The study determined five clusters of patients with HF labelled early onset, late onset, atrial fibrillation-related, metabolic and cardiometabolic, which might inform etiological research, clinical risk prediction and the design of HF trials. Additionally, using supervised machine learning, the authors developed a prediction model for routine clinical use with an online risk calculator available for patients and clinicians enabling evaluation of effectiveness and cost-effectiveness [37].

In conclusion, the use of advanced risk prediction models, which utilize technologies such as artificial intelligence and machine learning, alongside traditional risk prediction methods that rely on patient data, routine clinical laboratory test results and diagnostic information, has significantly improved the accuracy of identifying individuals at high risk for CVD or HF, as well as predicting short-term mortality. But a major clinical and research need still remains to identify novel biomarkers and approaches, to enhance CVD and HF

risk prediction and mitigate the impact of these conditions on the global population.

DNA methylation in CVD and HF

DNA methylation (DNAm) is a well-studied epigenetic modification, involving the addition of a methyl (CH3) group to a cytosine base, primarily in cytosine-guanine dinucleotides (CpGs) [38]. Traditionally, unmethylated CpGs are associated with gene activity, while CpG methylation is linked to gene silencing [38]. The role of DNAm in human diseases has been widely recognized [39]. Many recent studies have also investigated the impact of DNAm and CVD, including coronary heart disease (CHD), MI, stroke, HF, hypertension and other CVDs [40–54], and there is now growing evidence supporting strong link between DNAm changes, CVD and HF [45]. DNAm is also an attractive candidate for use as a disease biomarker [38, 55–57]. CpG modifications are relatively stable biochemical changes, easily detectable not only in tissues but also in blood, serum, plasma and cell-free DNA [55–57]. Furthermore, their dynamic and potentially reversible nature in response to biological and environmental factors makes them a valuable source for predicting response to therapy and prognosis of outcomes [55–57]. Currently, FDA-approved kits detecting DNAm biomarkers for cancers are commercially available [57]. The advancement of methylation array technology has also made it possible to generate prediction models called DNAm risk scores (MRS) [58]. MRS, based on transferring genetic risk score approaches to DNAm, are defined as weighted sums of an individual's methylation markers' beta values from a pre-selected number of CpG sites [58]. The most popular MRS prediction models are the epigenetic clocks, which are predictors of biological age [59–64]. Epigenetic clocks can be categorized based on the criteria for selecting CpG sites and the specific focus of their training data [54–59]. In particular, the first-generation epigenetic clocks, Horvath's and Hannum's, are known as chronological age-trained clocks [59, 60]. At variance, the second-generation epigenetic clocks, DNAm PhenoAge, and DNAm GrimAge, are also called mortality-trained clocks [61, 62]. Also, very recently, a third-generation epigenetic clock was developed by Belsky et al. [63]. In addition to age prediction, MRSs have been applied to predict individual risks of disease or treatment success [58], and models based on DNAm have recently been developed for CVD and HF risk prediction [65–71]. In the following sections, current information on these subjects will be presented and implications of DNAm for improving clinical risk prediction of CVD and HF discussed.

DNAm, CVD and risk prediction

DNA methylome investigations through epigenome-wide association approaches have deeply affected our CVD understanding and enabled the identification of novel biomarkers [40–43]. In 2015, Guarrera et al. were among the first who unveiled distinct DNAm changes linked to CVD [40]. Their seminal study, comparing MI and matched control individuals from the Italian European Prospective Investigation into Cancer and Nutrition (EPICOR study) and the Dutch EPIC (EPIC-NL) cohorts, revealed distinct methylation profiles in cases and controls. Differentially methylated regions (DMR) at the *ZBTB12* gene and *LINE-1* elements were found in white blood cells several years before the MI event, indicating that DNAm may serve as an independent marker of CV risk [40]. One year later, Rask-Andersen et al. investigated the DNA methylome in blood DNA from participants in the Northern Sweden Population Health Study (NSPHS) which also included cases of MI and identified 211 CpG sites (representing 196 genes) in individuals with a positive history of MI [41]. Such genes included *RYR2* and *KCNN1*, which are related to cardiac function, *NMNAT2*, *FMNL2*, *MEIS1*, *WNT7A*, *HAND2*, *TBX18*, *LMOD2*, *SOX17*, *FGF1* and *OVol1* which are involved in cardiogenesis, and *EPHA2*, *DYSF*, *SFRP4*, *NRG1*, *BNIP3*, *GDF15* and *MLC1*, which provides cardio-protection following ischaemic events or reperfusion injury [41]. In 2017, Li and colleagues reported a further analysis of DNAm in blood DNA from patients with Acute Coronary Syndrome (ACS) and from control subjects [42]. They found a strong connection between ACS and methylation at 47 CpG sites. Approximately 62% of which also showed significant correlations with the expression levels of known genes. Additionally, they found associations with smoking and low-density lipoprotein (LDL) cholesterol and identified pathways related to atherogenic signalling and adaptive immune response [42]. A few years later, Westerman et al. conducted DNAm analyses in the Women's Health Initiative (WHI) and FHS Offspring Cohorts to find more reliable epigenetic biomarkers for CV risk [43]. They identified two epigenetic modules whose activation correlated with CVD risk. These modules serve as a molecular indicator of cumulative CV risk factor exposure, thus improving clinical risk prediction. Additionally, they reported three regions associated with the genes *SLC9A1*, *SLC1A5* and *TNRC6C* showing methylation patterns associated with CVD risk. Also, a single CpG site in *SLC1A5* revealed a direct causal relationship with CHD [43]. In the same year, Fernández-Sanlés et al. discovered specific CpG sites linked to acute myocardial infarction (AMI) [44]. They identified 12 CpG sites that were duplicated in various independent cohorts with incident coronary and CVD, with 4 out of the 12 also

associating with incident CHD. These CpGs were labelled cg05575921 (*AHRR*), cg25769469 (*PTCD2*), cg21566642 (intergenic) and cg04988978 (*MPO*) [44]. Very recently, Krolevets et al., through a systematic review conducted following PRISMA guidelines, examined DNAm in CVD and created a database containing 74,580 unique CpG sites [45]. Out of these, 1452 CpG sites were validated in two or more publications and 441 CpG in three or more publications. In addition, two CpG sites were validated in six or more publications, cg01656216, located close to the *ZNF438* gene (associated with vascular disease and epigenetic age), and cg03636183, close to *F2RL3* gene linked to CHD, MI, smoking and air pollution. The most frequently reported genes were *TEAD1* and *PTPRN2* which were associated with outcomes ranging from vascular to cardiac disease. Also, STRING analysis revealed significant protein–protein interactions between the products of the differentially methylated genes, suggesting that dysregulation of the protein network contributes to CVD [45].

In parallel with research investigating the relationship between DNAm at specific sites and CVD in humans, many methylation-based disease risk scores (MRS) have been developed to predict CVD risk [65–69]. DNAm age has also been utilized to achieve the same goal. In 2020, Westerman et al. generated a new epigenomic risk score for CVD [65]. In this investigation, a cross-study learner (CSL) model by training time-to-event elastic net regressions was initially developed on three existing DNAm datasets generated using the BeadChip array technology. These datasets were obtained from three independent cohorts, namely the Women's Health Initiative (WHI) with 2023 participants, the FHS Offspring Cohort with 484 participants and the Lothian Birth Cohorts (LBC) with 818 participants. The CVD events in these cohorts were 1009, 125 and 297, respectively. Scores from these models were aggregated through a "stacking" method and the resulting ensemble CLS model was validated in an additional group of participants from FHS (validation cohort, $n=2103$, 180 cases). The CSL model showed strong associations with incident CVD in an unadjusted analysis (Hazard ratio, HR = 1.58), which was just partially attenuated by adjustment for standard covariates (age, sex and estimated cell type fractions; HR = 1.28) as well as CVD risk factors (HR = 1.29). The CSL model was found to be associated with the time-to-event of CVD in the FHS cohort (HR per SD = 1.28). It was also able to predict the status of MI in the Registre Gironí del COR (REGICOR) cohort (Odds Ratio per SD = 2.14). The CSL model showed enhanced ability to discriminate epigenetic risks in individuals classified at lower risk based on traditional metrics of CVD risk. This suggests that the CSL model is useful in identifying higher-risk individuals who would not have otherwise been detected by other risk metrics [65]. In 2022, Cappozzo et al. adopted a two-step approach and developed the DNAmCVDscore, a combined blood DNAm biomarker for predicting future CV events trained on CVD-specific risk factors [66]. In the first phase of the study, by using the LASSO (Least Absolute Shrinkage and Selection Operator) algorithm, nine novel DNAm surrogates for the CVD risk factors, i.e. BMI, CRP, fasting glucose and insulin, HDL cholesterol, triglycerides, PAI-1, platelet tissue factor (CD142) and systolic BP, have been used on the training set [participants from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort; EPIC Italy; n = 1803] and validated on a testing set [made up of participants from four independent cohorts, the EXPOsOMICS CVD ($n=315$), the Understanding Society ($n=1174$), the Irish longitudinal study on aging (TILDA; $n=490$) and the GSE174818 ($n=127$)]. In the second phase of the study, by adopting the elastic net regression model, the DNAmCVDscore has been developed, based on 10 DNAm surrogates (fasting glucose, HDL cholesterol, systolic BP, smoking pack-years, lead exposure and blood levels of PAI-1, CRP, SKR3, HGF and GDF15 proteins) regressed against the time from study recruitment to the CV event in the EPIC Italy ($n=1803$; $n=295$ CVD events during follow-up). Its prediction performance at different time points has been validated on the participants from the EXPOsOMICS CVD ($n=315$), the Northern Ireland Cohort for the Longitudinal Study of Ageing (NICOLA; $n=1728$) and The Health and Retirement Study (HRS; $n=2146$) using adjusted logistic regression models. Of note, the DNAmCVDscore revealed a higher ROC-AUC for short-term CV events than for long-term CVD and outperforms for short-term CVD risk (AUC from 0.71 to 0.85 for follow-up time at 7 years or less) the previously developed epigenetic-based scores for CVD risk, MRS (AUC from 0.67 to 0.72) and DNAmGrimAge (AUC from 0.71 to 0.84) and the traditional CVD risk algorithm SCORE2 (AUC from 0.68 to 0.75). Also, the DNAmCVDscore improves the predictive accuracy of SCORE2 for the entire time horizon considered in the study (follow-up time from 2 to 18 years), indicating that epigenetic-based biomarkers complement the information provided by traditional CVD risk factors [66].

DNAm can also capture individual protein concentration differences [72]. Protein epigenetic scores (EpiScores) are methylation scores derived from models of protein concentrations obtained by linear regression to be used as proxies for protein levels [72]. Also, EpiScores are more stable over time and may be more strongly associated with disease outcomes than individual protein measurements [72]. Recently, 109 EpiScores for circulating protein levels, where DNAm patterns explained

between 1 and 58% of the protein variation levels, have been developed. These episcores were associated with the time to diagnosis for different leading causes of morbidity and mortality, including CVD [72]. Chybowska et al. have recently developed a composite EpiScores for circulating protein levels associated with CVD risk independent of traditional risk factors [67]. Specifically, the study tested, through a series of Cox proportional hazard (PH) models, whether 109 EpiScores for circulating protein levels identified by Gadd et al. were associated with CVD risk over 16 years of follow-up of analysed data from ≥ 12,657 participants in the Generation Scotland (GS) cohort. This work revealed that individual EpiScores for 65 circulating proteins were associated with long-term risk of CVD independently of the clinical risk prediction assessed with ASSIGN [73] and the concentration of the cardiac Troponin I (cTnI). The most significant EpiScores reflected the concentration of CRP (HR = 1.23) and MMP12 (HR = 1.13), whose elevated levels were associated with increased hazard of CVD, and of NOTCH1 (HR = 0.84) and OMD (HR = 0.87), whose higher levels were associated with a decreased hazard of CVD. In a second phase of the study, using the modelling techniques, COX PH elastic net and random survival forest, the composite CVD EpiScore, based on 45 protein EpiScores, has been developed. This further work revealed that the CVD EpiScore is a significant predictor of CVD risk independent of ASSIGN and the concentration of cTnI (HR = 1.32) [67]. Also, the CVD EpiScore outperforms the null model containing age, sex, ASSIGN and cTnI both in a 10-year elastic net prediction and in a random survival forest-based analysis [67].

The hypothesis that DNAm age mediates the associations between CV risk factors, CVD and cardiac age and may enable risk prediction has also been explored. In 2023, Topriceanu et al. studied how DNAm age is related to A-ECG heart age and CV risk factors [68]. In this study DNAm age derived from first- (DNAm AgeHannum and AgeHorvath) and second- (PhenoAge and GrimAge) generation DNAm age biomarkers and their corresponding AgeAccel (linearly regressed on chronological age) [59–62] as well as the cardiac age derived from the A-ECG- and the DNN ECG-based heart age scores [22, 23] were calculated in 498 participants (range age, 60–64 years) of the Medical Research Council (MRC) British National Survey of Health and Development (NSHD) study, which included data prospectively collected from each subject. Also, using generalized linear models, associations of the derived epigenetic age and AgeAccel, the ECG-based heart age scores and the biological cardiometabolic risk factors (BMI, hypertension, diabetes, high cholesterol, previous CVD and any CV risk factor) were tested. These analyses revealed that by the age of 60, individuals with accelerated DNAm appear to have older, weaker and more electrically impaired heart function. Also, the association between CV risk factors and ECG-based cardiac ages and disease scores is partly mediated by the second-generation DNAm AgeAccel biomarkers. Indeed, AgeAccelPheno is a partial mediator for diabetes (average causal mediation effects, ACME = 0.23 years), for high cholesterol (ACME = 0.34 years) and for any CVD risk factor (ACME = 0.34 years). Similarly, AgeAccelGrim mediates ≈30% of the relationship between diabetes or high cholesterol and the DNN ECG-based heart age. Also, when exploring the link between cardiometabolic risk factors and the A-ECG-based LV electrical remodelling (LVER) and LV systolic dysfunction (LVSD) scores, the AgeAccelPheno or AgeAccelGrim mediates 10–40% of the total effects [68]. Compared to the first-generation DNAm ages [59, 60], the second-generation DNAm ages integrate clinical and physiological prognostic DNAm biomarkers and have been developed to serve as better predictors of health span (DNAm PhenoAge) [61] and lifespan (DNAm GrimAge) [62]. Also, DNAmPheno includes CpG sites related to the immune system, inflammation and metabolism, which have been associated with accelerated cardiac aging and increased susceptibility to CVDs [61]. At the same time, DNAm GrimAge incorporates blood-based biomarkers related to extracellular matrix remodelling, which extensively affects LVSD and LVER [62]. Recently, Carbonneau et al. conducted a further study, involving 5682 participants from the FHS, to investigate the role of DNAm age in the relationship between CV health, CVD and all-cause mortality [69]. The study used four established scores for DNA methylation-based epigenetic age: the DunedinPACE Score [63], the PhenoAge [61], the GrimAge [62] and the DNAmTL [64]. Assessment of CV health was achieved using the Life's Essential 8 (LE8) score, a metric composed of 8 factors, including diet, physical activity, nicotine exposure, sleep health, BMI, blood lipid levels, blood glucose levels and BP [74]. The study found that an increase of 1 standard deviation (equivalent to 13 points) in the LE8 score was associated with a 0.39 standard deviation lower DunedinPACE Score, a 0.42 standard deviation lower GrimAge, a 0.15 lower PhenoAge and a 0.10 higher DNAmTL. Furthermore, a 1 standard deviation increase in the LE8 score was correlated with a 35% lower risk of developing CVD, a 36% lower risk of CVD-specific mortality and a 29% lower risk of all-cause mortality. These associations were partly influenced by DNAm age biomarkers like GrimAge and DunedinPACE scores. Mediation analyses for the DunedinPACE and GrimAge scores revealed that the mean proportions of mediation were 14% and 21% for incident CVD, 14% and 21% for CVD-related mortality and 42% and 65% for all-cause mortality, respectively

[69]. However, using DNAm age as a proxy for cardiac aging is not always optimal. Pavanello et al. recently reported that the biological age of the heart, measured as DNAmAge, is consistently younger than chronological age [75]. In 2023, by applying the Bekaert [76], Weidner [77] and the Zbiec-Piekarska [78] DNAm clocks, Mongelli et al., using blood and auricle samples from donors undergoing cardiac surgery for coronary aortic bypass graft (CABG; $n=289$) or aortic valve replacement (AVR, $n=94$), have reported that the heart is younger than the blood [70]. These authors found that while the Bekaert's DNAm age estimation adequately determines the individual DNAmAge in blood, it also provides inappropriate DeltaAge between chronological and heart biological age [70]. Assuming that the heart may have intrinsic features that influence the organ-specific aging process, Mongelli et al. designed new blood- and the first cardiac-specific clocks, now termed the Mongelli & Panunzi (M&P) cardiac and blood model. The two models were built by including three independent selection procedures, a stepwise regression, the LASSO and the recursive feature elimination (RFE) algorithms and take into account 31 specific CpG sites from six age-related genes, *ELOVL2*, *EDARADD*, *ITGA2B*, *ASPA*, *PDE4C* and *FHL2*. These new methods revealed consistency between chronological and biological age in the blood and heart (Chronological age = 66.5 ± 9.7 years; M&P blood-DNAmAge = 65.7 ± 7.6 years; M&P Cardiac-DNAmAge = 66.4 ± 7.4 years). Additionally, the M&P clocks detect within the AVR and CABG participant groups with decelerated, regular and accelerated biological age, and interestingly, the cardiac-specific M&P clock revealed that DeltaAge acceleration within the AVR patient's subgroup correlated with altered ventricular parameters, including LV diastolic and systolic volume [70]. Thus, DNAmAge may be adopted in diagnostic procedures to identify subpopulation of patients that require a more intense clinical follow-up. The studies reporting DNAm-based prediction models for CVD and heart age are summarized in Table 1.

DNAm, HF and risk prediction

Studies are also being published which reveal the association between DNAm and HF [46, 50–54]. In 2017, Meder et al. conducted the first epigenome-wide association study in patients with HF using a multi-omic approach [46]. The study involved a detailed analysis of high-resolution epigenome-wide cardiac and blood DNA methylation as well as mRNA and whole-genome sequencing. This investigation was performed in a large group of extensively characterized patients with systolic HF following dilated cardiomyopathy (DCM). In the discovery cohort, the authors identified 59 differentially methylated CpG loci in the myocardium of patients with DCM ($n=41$) compared to patients without DCM ($n=31$). Among these loci, 30 were hypomethylated and 29 were hypermethylated, with 3 of them achieving epigenome-wide significance at $p \leq 5 \times 10^{-8}$, namely cg16318181, cg01977762 and cg23296652. Of note, 27 of the 59 loci, including the cg16318181 within the gene body of *CMSS1*, the 5′UTR region of *FILIP1L* and part of the promoter region of *miR-548G*, were also replicated in independent cohorts. Furthermore, when comparing the methylation changes from previous studies (34 loci) [47–49] with their dataset, the authors showed replicated DNAm changes in the genes *LY75*, *PTGES*, *CTNNAL1*, *TNFSF14*, *MRPL16* and *KIF17*. These findings strengthened the association of HF with reproducible DNAm modifications. The study also confirmed known age-dependent patterns in CpG islands in *ELOVL2*, *FHL2* and *PENK* genes using DNA from whole-blood samples of the cohort. Two of the significantly confirmed CpG sites were found to be linked to the expression of neighbouring genes in both the discovery and validation groups. DNAm of the cg25838968 linked to *PLXNA2* gene and DNAm of the cg14523204 associated with *RGS3* gene were found to be differentially expressed in DCM. In addition, the comparison between the methylation patterns from myocardial tissue and peripheral blood in the screening and replication cohorts revealed that the two hypomethylated CpGs, cg24884140 (*B9D*) and cg12115081 (*doublecortin-like kinase 2*), and the hypermethylated CpG, cg25943276 (*neurotrimin*), in DCM, significantly overlap between tissue and blood. Also, the combination of these three markers exhibited an excellent performance as a blood test for DCM, with an accuracy of 91.5% in the discovery group and 86.9% in the validation group which exceeded the accuracy of NT-proBNP (85%), a commonly used biomarker of HF [46]. Two years later, Pepin et al. examined genome-wide cardiac DNAm in patients with end-stage HF to determine whether epigenetic reprogramming occurs in Ischaemic Cardiomyopathy (ICM) [50]. In this study, including biopsies of cardiac LV from HF patients classified as either ICM ($n=5$) or NICM ($n=6$), combined genome-wide gene expression and DNAm analyses reflect metabolic gene reprogramming. The 211 differently methylated CpGs corresponding to 124 differentially expressed genes mediate oxidative metabolic gene suppression via promoter hypermethylation of genes involved in electron transport, TCA cycle and fatty acid beta-oxidation, and regression to the foetal gene program through hypomethylation of anaerobic glycolytic genes. These authors further identified a potential regulator of cardiac DNAm, EZH2 and suggested a new mechanism by which HF may affect the expression of enzymes and

Table 1 DNAm-based prediction models for CVD, HF and Heart Age

Research article(s)	Prediction model(s)	Cohort(s)	Main finding(s)
Meder et al. [46]	3-marker methylation panel for HF caused by DCM	*Screening cohort:* patients with DCM ($n=41$) and clinical controls ($n=31$). Heart and blood samples *Replication cohort I:* Heart sample, patients with DCM ($n=18$) and healthy controls ($n=8$); Blood samples, patients with DCM ($n=9$) and healthy controls ($n=28$) *Replication cohort II:* Blood samples, patients with DCM ($n=82$) and healthy controls ($n=109$)	The 3-marker methylation panel exhibits excellent diagnostic performance for DCM and outperforms the NT-proBNP
Westerman et al. [65]	CVD Risk CSL	*Training cohorts*: participants from WHI ($n=2023$; CVD cases, $n=1009$), FHS-JHU ($n=484$; CVD cases, $n=125$), LBC ($n=818$; CVD cases, $n=297$), and FHS-UM ($n=2103$; CVD cases, $n=180$) *Replication cohort*: participants from REGICOR ($n=391$; CVD cases, $n=191$)	The CSL associates with CVD time-to-event, performs best in individuals with lower Framingham Risk Scores and predicts MI status
Cappozzo et al [66]	Composite biomarker predictive of CVD risk	*Training cohort*: participants from EPIC Italy ($n=1803$) *Validation cohorts*: participants from Understanding Society ($n=1174$), TILDA ($n=490$), EXPOsOMICS CVD ($n=315$), GSE174818 ($n=127$), HRS ($n=2146$) and NICOLA ($n=1728$)	The DNAmCVDscore shows high performance in predicting short-term CV events outperforming current state-of-the-art CVD prediction models based on traditional risk factors
Zhao et al (2022) [71]	Composite HFmeRisk score	*Training cohort*: participants from FHS Offspring cohort ($n=797$; HFpEF $n=59$) *Testing cohort:* participants from FHS Offspring cohort ($n=171$; HFpEF $n=32$)	The HFmeRisk score integrates both clinical and epigenetic features and outperforms models with clinical characteristics or DNAm alone, and published chronic HF risk prediction models
Tropiceanu et al [68]	DNAm Age scores (AgeHannum, AgeHorvath, PhenoAge, and GrimAge) relation to CV risk factors	*Study cohort:* participants from MRC NSHD ($n=498$)	By the age of 60, participants with accelerated DNAm have older, weaker, and more electrically impaired hearts
Mongelli et al [70]	Mongelli & Panunzi (M&P) cardiac and blood clocks	*Study cohort:* Patients undergoing cardiac surgery ($n=383$; CABG, $n=289$; AVR, $n=94$) *Training cohort*: $n=288$ *Testing cohort:* $n=95$	The M&P cardiac and blood clocks consist of 31 specific CpG sites and reveal similarity between chronological and biological age in the blood and heart
Chybowska et al [67]	Composite CVD EpiScore	*Study cohort*: participants from GS ($n=\geq 12{,}657$ CVD cases, ≥ 1274) *Training cohort*: GS ($n=6880$) *Testing cohort:* GS ($n=3659$)	The composite CVD EpiScore, based on 45 protein EpiScores, is a significant predictor of CVD risk independent of ASSIGN and cTnI concentration
Carbonneau et al [69]	DNAm Age scores (DunedinPACE, PhenoAge, GrimAge, and DNAmTL) relation to CV health, CVD and all-cause mortality	*Study cohort:* participants from FHS ($n=5682$)	DNAm Age scores mediate the associations between the LE8 score and incident CVD, CVD-specific mortality, and all-cause mortality

AVR, aortic valve replacement; CABG, coronary aortic bypass graft surgery; CSL, cross-study learner model; CVD, cardiovascular disease; DCM, dilated cardiomyopathy; EPIC, European Prospective Investigation into Cancer and Nutrition; EXPOsOMICS CVD, case–control study nested in the EPIC Italy cohort; FHS, Framingham Heart Study; FHS-JHU, Framingham Heart Study (Johns Hopkins University); FHS-UM, Framingham Heart Study (University of Minnesota); GS, Generation Scotland; HF, Heart Failure; HRS, The Health and Retirement Study; LBC, Lothian Birth Cohorts 1936; MRC NSHD, the Medical Research Council National Survey of Health of Development; NICOLA, Northern Ireland Cohort for the Longitudinal Study of Ageing; REGICOR, Registre Gironí del COR; TILDA, The Irish longitudinal study on ageing; Understanding Society, The United Kingdom Household Panel Study; WHI, Women's Health Initiative

regulators of cardiac metabolism, such as KLF15 [50]. In the same year, Pepin et al. also examined whether the DNA methylome and transcriptome are reprogrammed in HF by comparing whole-genome data from cardiac LV biopsies of patients with end-stage HF ($n=7$) and nonfailing donor hearts ($n=3$) [51]. These differential methylation studies revealed a diametric metabolic shift in cardiac genes, with hypermethylated gene promoters associated with mitochondrial compartmentalization and oxidative pathways and hypomethylated gene promoters enriched in genes involved in glycolysis and anaerobic metabolic processes. Also, epigenetic interference of *NRF1* via hypermethylation of its downstream promoter targets was identified. All these findings support cardiac reprogramming by epigenetic modifications of metabolic genes in HF [51]. Subsequently, Bain et al. adopted methyl-binding domain-capture sequencing in a cohort of 20 male patients, 10 of which with multivessel CAD and HF. The authors identified 68 DMRs in HF, 48 of which occurring within gene bodies and 25 located near enhancer elements [52]. Of the HF-associated DMRs, genes of particular interest as novel candidate markers of HF were *HDAC9*, *JARID2* and *GREM1*, with reduced methylation, and *PDSS2*, with increased methylation [52]. In 2023, Liao et al. determined the DNA methylation profile of myocardial tissue in patients with end-stage cardiomyopathy [53]. The study included 36 patients with end-stage HF who underwent LV Assist Device (LVAD) implantation at Columbia University Irving Medical Center. Twelve of these patients had ICM, 24 nonischaemic dilated cardiomyopathy (NICM), and 7 were nonfailing (NF). The genome-wide DNAm analysis identified a total of 2,079 differentially methylated positions (DMPs) in the myocardium of the patients with ICM. Among these, 625 DMPs were found to be hypermethylated and 1,454 were hypomethylated. In addition, 261 DMPs were identified in the myocardium of patients with NICM, with 117 DMPs being hypermethylated and 144 hypomethylated. Also, 192 HF DMPs were common to both patients with ICM and NICM and were either concordantly hypomethylated ($n=125$) or hypermethylated ($n=67$). The study also found minimal reversibility of myocardial DNAm with LVAD support, with only 35 CpG sites being common in HF and reverse remodelling, though all methylated in opposite directions. Additionally, the analysis of DNAm with gene expression in the failing human heart revealed several protein-coding genes that are hypomethylated and upregulated (*HTRA1*, *FBXO16*, *EFCAB13* and *AKAP13*) or hypermethylated and downregulated (*TBX3*) in HF. Furthermore, the long noncoding RNA *LINC00881* was identified to feature epigenetic and transcriptional dysregulation in patients with ICM and NICM. *LINC00881,* transcribed from a cardiac-specific super-enhancer region with abundant expression levels in the adult human heart, is an upstream regulator of the sarcomere and calcium channel gene expression, including *MYH6*, *CACNA1C* and *RYR2*, and its knockdown reduces peak calcium amplitude in the beating human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) [53]. Qi et al. very recently discovered a link between DNAm at the *iodothyronine deiodinase 3* gene promoter region fragment FA27 (*DIO3-FA27*), biochemical markers and HF [54]. In this study involving 20 patients diagnosed with HF, a quantitative DNAm analysis on *DIO3-FA27* promoter through a Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF–MS), revealed lower DNAm of the CpG_11.12 and CpG_23.24 in HF patients in NYHA class III/IV compared to HF patients in class I/II. Also, a restrictive cubic spline model revealed that the DNAm levels of CpG_11.12 and CpG_23.24 were associated with coagulation, liver and renal function biomarkers, suggesting that biochemical perturbations, combined with certain levels of *DIO3-FA27* promoter DNAm, may worsen the prognosis of patients with HF [54].

In 2022, a prediction score, termed HFmeRisk, combining multi-omic data interactions through end-to-end machine learning models, has been developed to explore the interaction between DNAm and clinical features in predicting the early onset of HFpEF [71]. In particular, in this study Zhao et al. included participants free of chronic HF (CHF) at baseline in FHS Offspring Cohort exam 8, with a specific diagnosis of HFpEF or no-CHF and a follow-up of 8 years, with complete medical information and qualified DNAm data (training set cohort: HFpEF; $n=59$; no-CHF, $n=738$; testing set cohort: HFpEF; $n=32$; no-CHF, $n=139$). This study involved the use of the LASSO and XGBoost (Extreme Gradient Boosting) algorithms to perform feature selection and of the DeepFM (Factorization-Machine based neural network-based recommender system) algorithm to build the prediction model. The developed HFmeRisk framework includes 25 specific CpGs and five electronic health record (EHR) variables (age, diuretic use, BMI, albuminuria and serum creatinine) and accurately predicts the early risk of HFpEF. The HFmeRisk achieved an AUC of 0.90, which outperformed models with the five clinical characteristics (AUC = 0.78) or the DNAm levels of the 25 CpGs alone (AUC = 0.65), the Hannum 26 age-related CpG (AUC = 0.65) [60] and other benchmark machine learning models (AUCs from 0.63 to 0.83). Also, the HFmeRisk featured the best performance compared to prediction models fed with other omics, such as the EHR + RNA model (AUC = 0.78) and the EHR + microRNA model (AUC = 0.80), indicating that DNAm is more suitable than RNA to predict CHF risk. This investigation

further demonstrated that CpG sites in the HFmeRisk have key functions for pathways related to the causal mechanism of HFpEF, including intercellular signalling and interaction, amino acid metabolism, transport and activation, and were related to genes associated with risk factors for HF such as BMI, systolic and diastolic BP, EF and T2D [71]. The studies reporting DNAm based-prediction models for HF are summarized in Table 1.

Future directions: histone modifications and noncoding RNA for risk prediction in CVD and HF?

Concurrent with the evidence revealing the presence of DNAm changes in CVD and HF, many studies have also reported the role of post-translational modifications of histone tails and post-transcriptional regulation of gene expression by noncoding RNA (ncRNAs) in the regulation of myocardial and vascular function in health and disease [79]. The sections below will briefly discuss the implications of these abnormalities in CVD and HF and their potential use for improving clinical risk prediction of these diseases.

Histone modifications in CVD and HF

Histone modification is one of the most important and complex epigenetic regulatory mechanisms in eukaryotes [80]. Histone post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitylation, and the less common ribosylation, sumoylation and citrullination, are finely regulated by histone modifying enzymes, which catalyse the addition or removal of covalent modifications [80]. In 2013, Papait et al. provided compelling evidence that the histone modification landscape is a key determinant of gene expression reprogramming in cardiac hypertrophy [81]. In their work these authors used a multi-omic approach, which involved a genome-wide map of seven histone modifications (H3K9ac, H3K27ac, H3K4me3, H3K79me2, H3K9me2, H3K9me3 and H3K27me3) and a transcriptome analysis of gene expression. Their study in adult mouse cardiomyocytes exposed to a hypertrophic stimulus in vivo, revealed that 9.1% of the genome in hypertrophic cardiomyocytes experienced a change in the distribution of at least one histone mark. The sites showing differential distribution of H3K9ac, H3K27ac or H3K4me3 were found to be mainly associated with the regulatory regions of genes involved in heart function or the epigenetic control of gene expression. On the other hand, sites with altered H3K79me2, H3K9me2, H3K9me3 or H3K27me3 profiles were predominantly linked to genes that regulate signal transduction, as well as the organization and regulation of sarcomeric structure. Sites with differential distribution of H3K9ac, H3K4me3, H3K79me2 or H3K27me3 were associated with hypertrophic heart phenotypes. Finally, the authors identified 9,207 potential active enhancers whose activity was modulated. The analysis of the transcriptional network revealed that the myocyte enhancer factors MEF2C and MEF2A play a role in regulating enhancers during cardiac hypertrophy [81].

Among all histone post-translational modifications, methylation and acetylation and the role of their modifying enzymes were mainly studied and involved in the development of CVD and HF [82]. The histone trimethyllysine demethylase JMJD2A was found to feature increased expression in human patients with hypertrophic cardiomyopathy. In mice, this enzyme promotes cardiac hypertrophy in response to hypertrophic stimuli by binding to the promoter of FHL1 which leads to upregulation of *FHL1* expression and downregulation of H3K9 trimethylation [83]. Another example is the histone methyltransferase G9a, which has been reported to regulate cardiomyocyte homeostasis in the adult heart by mediating repression of key genes that regulate cardiomyocyte function through dimethylation of H3 lysine 9 and interaction with the catalytic subunit of polycomb repressive complex 2 [84]. The histone acetyltransferase p300 also plays an important role in cardiac development and HF [85, 86]. p300 controls the expression of *GATA4* during embryonic mouse cardiogenesis through the acetylation of H3K4, H3K9 and H3K27 in the *GATA4* promoter [85]. Additionally, p300 mediates the acetylation of the *SERCA2a* at lysine 492. In failing hearts, the acetylation of *SERCA2a* is significantly increased, leading to reduced SERCA2a activity. Importantly, reversing *SERCA2a* acetylation by pharmacological activation of the histone deacetylase SIRT1 can recover SERCA2a function and improve cardiac defects in failing hearts, offering a novel potential strategy for the treatment of HF [86].

Noncoding RNA in CVD and HF

ncRNAs, by definition, are a group of heterogeneous transcripts of different types and sizes that are not translated into proteins [87]. ncRNAs operate through various mechanisms to influence post-transcriptional regulation of expression of target genes and interact with each other, forming a complex and dynamic regulatory RNA network [87]. Also, ncRNAs are major regulators of various biological functions in different cell types and tissues [87]. Dysregulation of ncRNAs, particularly microRNAs (miRNAs), has been linked to human diseases, including CVD and HF [88–92]. For instance, *miR-1* is the most abundant miRNA in the heart and is specific to the heart and muscles. The levels of circulating cell-free *miR-1* increased in patients with AMI and showed a positive correlation with serum CK-MB levels [93]. Plasma *miR-1*, *miR-133a*, *miR-133b* and *miR-208b*

were linked to hs-TnT levels in a large group of patients with ACS. Patients who had experienced a heart attack had higher levels of *miR-1*, *miR-133a* and *miR-208b* compared to those with unstable angina. Also, *miR-133a* and *miR-208b* were significantly associated with the risk of death [94]. The levels of serum *miR-21* were reported to be significantly higher in HF patients than those in control subjects and were correlated with EF and BNP levels. Furthermore, *miR-21* has high levels of sensitivity and specificity for diagnosing HF [95]. Notably, numerous publications are reporting on circulating miRNAs as potential biomarkers for the diagnosis and prognosis of CVD and HF only this year [96–99]. Plasma expression of *miR-106a-5p* has been demonstrated to undergo downregulated in acute HF (AHF) [96]. In their work, including 127 AHF patients and 127 control individuals followed up for 1 year, Fei et al. showed that plasma *miR-106a-5p* is expressed at lower levels in AHF patients compared to controls and negatively correlates with NT-proBNP and CRP levels. Also, plasma *miR-106a-5p* level lower than the cut-off 0.655 aids in the diagnosis of AHF and level lower than 0.544 predicts poor prognosis in AHF patients [96]. The diagnostic and prognostic significance of *miR-320a-3p* has been reported in patients with CHF [97]. In this study, 103 patients with CHF and 95 healthy controls were examined. The levels of serum *miR-320a-3p* were elevated in CHF patients, and the levels of BNP and LVEF were found to be positively and negatively correlated with *miR-320a-3p*, respectively. The high diagnostic accuracy of *miR-320a-3p* for CHF was indicated by a ROC-AUC value of 0.866. Survival curve and Cox analysis also revealed that high expression of *miR-320a-3p* was linked to poor prognosis in CHF patients [97]. In the same year, Marchegiani et al. identified prognostic minimally invasive miRNA biomarkers capable to assess mortality risk in patients with cardiovascular multimorbidity [98]. Their study, involving 246 hospitalized geriatric patients (median age 86 years) followed for up to 24 months, revealed that lower circulating levels of *miR-17* and miR-126-3p were significantly associated with increased short- and medium-term mortality risk. This finding helps in identifying patients at higher risk of mortality. Specifically, patients with the lowest levels of *miR-17* upon hospital admission had a higher risk of mortality at 31 days, while those with the lowest levels of *miR-126-3p* had a higher risk of mortality at 24-month follow-up. Conversely, high expression levels of *miR-17* and *miR-126-3p* at admission were linked to better prognosis [98]. A miRNA signatures of CVD and its risk factors at the population level in middle-aged and older adults have been also reported [99]. In their study, Karlin et al. measured plasma miRNA levels in 4440 participants of the FHS. Linear regression analyses were conducted to test associations of each miRNA with various risk factors. Additionally, prospective analyses were conducted to assess the associations of miRNAs with new-onset obesity, hypertension, T2D, CVD and all-cause mortality. In the FHS, *miR-193b-3p* and *miR-122-5p* were significantly associated with six CVD risk factors, and *miR-365a-3p*, *miR-194-5p*, *miR-192-5p* and *miR-193a-5p* were each associated with five risk factors. Also, *miR-193b-3p*, *miR-194-5p* and *miR-193a-5p* were each associated with two or more risk factors in the 1999 participants of the Rotterdam Study. Finally, prospective analyses revealed that *miR-193a-5p*, *miR-192-5p*, *miR-122-5p* and *miR-193b-3p*, along with *miR-320e*, *miR-210-3p*, *miR-34a-5p* and *miR-301a-3p*, were associated with all-cause mortality in the FHS [99].

Histone post-translational modifications on specific target regions are potential sources of biomarkers that could be useful for CVD and HF assessment. However, some issues still limit their application in clinical practice. For instance, histone modifications have mainly been reported in cardiomyocytes or heart samples [81–86]. It remains to be demonstrated that the same histone modifications specific to the myocardium might be found in circulating leukocytes. Additionally, technical challenges, such as sample preparation, DNA integrity, antibody specificity and cross-reactivity, must be overcome. In contrast to histone modifications, there is more robust evidence supporting the potential of ncRNAs as diagnostic and prognostic biomarkers for CVD and HF. ncRNAs can be isolated from tissue biopsies and liquid specimens such as blood, serum, plasma and extracellular vesicles, and be easily detected and analysed using standardized techniques including quantitative real-time PCR, droplet digital PCR or RNA sequencing [87]. Additionally, circulating ncRNAs are remarkably stable in blood, and specific miRNAs that are unique to cardiac tissue have been tested as circulating biomarkers [87]. Also in this case, however, several challenges still need to be addressed. For example, changes in the levels of the same circulating ncRNAs have been reported in different CVD and non-cardiac diseases [87]. Also, there are conflicting observations regarding the quantification of ncRNAs among studies, highlighting the need for standardized procedures and protocols for material management, ncRNA isolation and quantification [87].

Conclusions

This review has examined most recent findings from the investigation of DNAm in CVD and HF in humans. Methylated CpG sites identified in CVD and HF belong to genes that can impact cardiac and vascular function. Also, many CpG sites have been adopted to design specific prediction algorithms for CVD and HF, with similar

or even enhanced performance compared to existing scores based on clinical risk factors alone. For instance, Westerman's CSL model associates with CVD time-to-event and predicts MI. It also helps identify high-risk individuals who may not be identified by other risk metrics [65]. Cappozzo's DNAmCVDscore, which is based on 10 DNA methylation surrogate biomarkers, performs better in predicting short-term CVD risk compared to the traditional CVD risk algorithm SCORE2. It also improves the predictive accuracy of SCORE2 for follow-up periods ranging from 2 to 18 years [66]. Similarly, Chybowska's CVD EpiScore, a composite model based on 45 circulating proteins, is associated with CVD risk independently of traditional risk factors. It also outperforms a null model containing age, sex, ASSIGN and cTnI in predicting CVD risk over a 10-year period [67]. Finally, Zhao's HFmeRisk, a deep learning model that incorporates DNAm biomarkers and clinical features, exceeds risk scores based solely on clinical findings or CpG methylation and other benchmark machine learning models in predicting the early risk of HFpEF [71].

While the field of CVD epigenetics is rapidly advancing, there still remains an urgent need of standardized procedures, the lack of which hinder comprehensive comparisons and synthesis of the available information, limiting the application of these epigenetic scores in current clinical practice. DNAm is, indeed, commonly identified using different profiling procedures, such as sequencing techniques or methylation arrays [100, 101]. Sequencing data offer more comprehensive CpG methylation information, but the present high cost of whole-genome bisulphite sequencing platforms makes large-scale research impractical [101]. Methylation arrays, on the other hand, offer a reasonable compromise as the approach is cheaper than whole-genome bisulphite sequencing and covers a large portion of the methylated genome [45, 101]. At the present, the most common methods for characterizing DNAm in humans have been the 450 k methylation array from Illumina, which measures methylation at around 450,000 CpG loci throughout the genome, and the updated 850k array, which covers almost twice as many CpG loci as the 450k array [102, 103]. However, since the two arrays do not cover the same set of CpGs, they only allow limited comparisons [103]. However, technology is constantly evolving and generation of custom-designed methylation arrays comprehensive of all CpGs relevant to a specific disease will represent a relatively cheaper solution and enable the screening of large sample sets. Furthermore, the current epigenetic prediction scores of CVD and HF have only been validated in a limited number of individuals and should be replicated across different human ethnicities. Refining DNAm prediction models should be also achieved by re-training the model after increasing the sample size by combining data from multiple cohorts and countries and using updated analytical methods. Replicating these results in more extensive and diverse populations will improve finding validation and eventually help modelling ethnicity-specific prediction scores [66–69]. Further limitation is determined by the fact that, at the present, existing epigenetic prediction models mainly rely on DNAm assessments obtained from blood, which restricts the exploration to simply establishing correlations between the identified CpG modifications in CVD and HF. CpG methylations in blood may be different from those in the heart or blood vessel cells. Accordingly, it is crucial to provide evidence of consistent results in blood and the target tissue or isolated cells to establish the biological relevance of the differential DNAm patterns in health and diseases. Finally, the current epigenetic scores are based on CpG biomarkers, while DNAm is only one of several potentially relevant epigenetic modifications. Future investigations might reveal new epigenetic markers. For instance, as mentioned in the previous paragraph, miRNAs have already been related to the development and progression of CVDs, including HF, and circulating miRNAs, which are easily detectable in peripheral blood, show potential applicability as diagnostic or prognostic biomarkers for CVD and HF [88–92]. Understanding epigenetic heart aging will also improve risk prediction models for these diseases.

In the near future, research work is expected to lead to the development of epigenetic prediction models that focus on specific heart or vascular diseases (such as MI, CAD, aortic atherosclerosis, stroke or peripheral artery disease) and help identifying individuals at high risk early in life and better stratify patients with different disease trajectories and prognosis. Also, advances in artificial intelligence applied to CV medicine and the development of new machine learning algorithms with improved performances, specifically designed to integrate data from DNA methylomics, other sources and patient clinical information will help in personalizing risk prediction for CVD and HF. The following approaches will enhance accuracy of diagnosis and effectiveness of treatment through personalized, risk-focused targeted therapies. They will also empower patients to take control of their health, enable early detection of CVD and HF, develop more cost-effective strategies and identify new care pathways.

Abbreviations

AVR	Aortic valve replacement
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
BP	Blood pressure
CABG	Coronary aortic bypass graft
CAD	Coronary artery disease

CpG	Cytosine-guanine dinucleotide
cTnI	Cardiac Troponin I
CVD	Cardiovascular disease
DCM	Dilated cardiomyopathy
DMP	Differentially methylated position
DNAm	DNA methylation
ECG	Electrocardiography
HDL	High-density lipoprotein cholesterol
HER	Electronic health record
HF	Heart failure
HFmrEF	Heart failure with mildly reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
hs-TnT	High-sensitive troponin T
ICM	Ischaemic Cardiomyopathy
LDL	Low-density lipoprotein cholesterol
LV	Left ventricular
LVH	LV hypertrophy
MI	Myocardial infarction
miRNA	MicroRNA
MRS	Methylation-based disease risk scores
ncRNA	Noncoding RNA
QoL	Quality of life
T2D	Type 2 diabetes

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Availability of data and materials
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Declarations

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