The Therapeutic Potential of Epigenome-modifying drugs in Cardiometabolic disease

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Abstract

Purpose of review:

Cardiometabolic disorders, including cardiovascular disease, obesity and type 2 diabetes are an extreme burden on the health of individuals and societies worldwide. The combination of genetic susceptibility, environmental, and lifestyle factors drive disease development. Therefore, understanding the exact molecular changes occuring in their pathogenesis brings not only insights into mechanism, but also potential novel therapeutic angles. Epigenetic changes - the mitotically heritable chemical marks or packaging of DNA that influence gene expression without changing the genetic code itself – have been identified as highly compelling targets. Therapeutic success modulating the epigenome has initially come in cancer, but is now expanding into non-malignant diseases. Here, we discuss the different epigenetic mechanisms implicated in cardiometabolic disease and the advancing science of epigenetic therapeutics for these disorders.

Recent findings:

Disruption of epigenetic networks can be causative in cardiometabolic diseases, including for example, endothelial dysfunction and vascular ageing. Epigenomic changes may also influence or indicate known risk factors, including hypertension, inflammation and dyslipidemia. The dynamic plasticity that characterizes the epigenome lends itself to therapeutic manipulation with many epigenetic drugs now under clinical trials and others have already been approved for medical use. Major epigenetic inhibitor classes include: DNA Methyltransferase inhibitors (DNMTi); Histone Deacetylase inhibitors (HDACi); Isocitrate Dehydrogenase inhibitors (IDHi); Bromodomain and the extra-terminal motif proteins inhibitors

(BETi); & EZH2 inhibitors (EZH2i); with natural and synthetic examples of these possessing potential cardiometabolic effects. For example, there is accumulating evidence for the BETi Apabetalone to reduce vascular inflammation, thereby decreasing cardiovascular events, and the HDACi Sodium Butyrate to suppress experimentally inflammatory cytokines in atherosclerosis.

Summary:

Despite challenges in the development and application of epigenetic drugs, the achievements in oncology and increasing experimental evidence indicate the clinical utility of targeting the readers, writers and erasers of the epigenome. Therefore, precise modifying of epigenetic information is likely to be a key player in the future of personalized therapeutic approaches in individuals with cardiometabolic disorders.

Keywords:

Epigenomics, Epigenetics, Cardiovascular Disease, Type 2 Diabetes, Obesity, Hypertension, Hyperlipidaemia, Inflammation, Atheroscelerosis, DNA methylation, Histone post-translational modifications.

Introduction

The burden of cardiometabolic disease is growing globally [1], and further significant improvements are needed in early prevention, prediction and detection to avoid the disease-related mortality and morbidity [2]. During the past two decades, the study of genomic variation has been highly successful in establishing the field of personalised medicine. International efforts have extensively described the human genome and analysed its variation in both healthy and diseased individuals. It is undoubtable that the knowledge gained from these genetic studies has paved the way to our understanding of the molecular basis of different human disorders. Nevertheless, whilst studies of genotype-phenotype relationships have brought many insights to researchers and clinicians with robust benefits to patients [3], this work has not been a complete panacea. Some observations do not follow the classic genetic heritability [4] and, furthermore, the integration of tissue-specific regulatory information brings an additional dimension to the intricacies of pathogenesis [5].

Epigenetics is a molecular explanation that bridges the gap between the static genome, developmental trajectories [6], and specialised cell-specific activity [7], as well as the complexity of environmental signals [8]. Epigenetic marks, in contrast to genetic mutations, do not alter the DNA sequence but rather alter the way cells use these DNA instructions [9]. Epigenetic signatures can be associated with lifestyle and environmental factors, during intrauterine, postnatal development, or across the entire lifecourse. Being modifiable, the epigenome provides an enticing opportunity to comprehend disease-related variation, and when causative, its possible reversion [4]. Many initiatives have sought to determine the changes in the epigenome of human disorders, including cardiometabolic disease, cancer, and neurodegenerative diseases. Whilst, many of these observed changes are not causally involved in the pathogenesis, robust associations are still potential disease biomarkers with clinical utility. Those epigenetic modifications that do display evidence of driving these conditions are targets for novel drugs to revert these defects. Therefore, epigenetics is emerging as a key player in the risk prediction, diagnosis, prognosis, as well as personalized treatment of cardiovascular diseases and cardiometabolic syndrome. This review highlights those epigenetic modifications discovered in relation to

cardiometabolic disease and explores the current state of the potential therapeutic use of epigenome-modifying drugs in these conditions. It also presents some of the challenges associated with pharmacological targeting of epigenetic networks.

1. The epigenome

In 1942, embryologist Conrad Waddington coined the term 'epigenetics', defining it as being the sum of developmental processes that lie between the genotype and the phenotype and dynamically connect them [10]. Although much conjecture has ensued regarding definitions following this [11], at its most straightforward, this term refers to the mitotically-heritable chemical modifications and packaging of genomic DNA leading to a change in the gene expression without altering the genetic sequence itself [12]. Fundamental to the packing mechanism is chromatin, a nucleoprotein-complex composed of DNA and histone proteins that make up the chromosome in eukaryotic cells [13]. It is the scaffold for packaging the whole genome and is also a dynamically adjusted structure that mirrors the regulatory signals responsible for various cell processes, including DNA replication, transcription, DNA repair and cell division [14]. Therefore, the epigenome is the sum of changes involved in chromatin modelling and post-translational histone tail modifications, which can at times be guided by non-coding RNA molecules, as well as direct modifications to the DNA itself [15].

1.1 DNA Modifications

The most common direct chemical addition to DNA is the classical epigenetic mark of DNA methylation. In eukaryotes, DNA methylation only targets cytosine residues (C-5 position of the pyrimidine ring in DNA). It is functionally involved in a range of regulatory processes [16], including different stable epigenetic suppression mechanisms, such as parent-of-origin imprinting, silencing of repetitive DNA and inactivation of the X chromosome [16]. The DNA methylome, or the genomic state of methylation, is strongly encoded by CpG density as well as the interaction of transcription factors and their sequence motifs [16]. Cytosine methylation occurs mainly at CpG dinucleotides (C followed by G in the 5' to 3' direction of the DNA strand) that are sparsely located or at repetitive elements, and it is usually not found in normal somatic cells where CpGs are densely packed

together (termed CpG islands). Methylated cytosine is susceptible to spontaneous deamination to thymine. This 5mC mutation causes a guanine-thymine mismatch, which is repaired in an error-prone manner [17]. This hypermutability means that CpG sequence context is reduced to ~20% of the expected level in the human genome [17] and ~30% of common human SNPs reside at disrupted CpG dinucleotides [18]. Recent analysis of vital loss-of-function intolerant genes identified that purifying selection acts to suppress germline methylation at their promoters to reduce the CpG mutation rate in these critical regulatory regions [19].

One group of enzymes that play an essential role in regulating DNA methylation is the DNA methyltransferase family (DNMT), which includes the maintenance DNMT1, and *de novo* DNMT3A and DNMT3B, as well as the regulatory factor DNMT3L [20]. DNMTs are the writers of DNA methylation (see Figure 1). They are highly conserved proteins that catalyze the addition of a methyl group from the methyl donor S-adenosylmethionine (SAM) to cytosine to form 5-methylcytosine [20,21]. DNA methylation removal can occur in an active or passive way. Active demethylation is mediated by the ten-eleven translocation (TET) enzymes [22]. The rare DNA modifications are steps in the active DNA demethylation process. TETs add a hydroxyl group to the methyl group of 5mC to convert it into its oxidized derivative 5-hydroxymethylcytosine (5hmC) and sequentially further to 5-formyl cytosine (5fC) and then 5- carboxylcytosine (5caC). A final step of base excision repair leads to the restoration of an unmodified cytosine [23]. On the other hand, the passive loss of a methyl group takes place due to a reduction of DNMT1 activity through replication [24]. Therefore, mutations within these epigenetic modifiers (DNMTs and TETs) can lead to a change in genome-wide methylation profile, resulting in global changes in the activation and repression pattern of downstream genes [25]. The extensive cancer sequencing projects over the last decade have identified an enrichment of oncogenic driver mutations in epigenomic modifier genes [13,26].

1.2 Post Translational Histone Tail Modifications

The N-terminal tails that project out from the core histones are exposed to different potential post-translational modifications [27]. Currently, more than ten varieties of histone modifications have been identified at different histone sites, and these modifications include acetylation, methylation, ubiquitination, and

phosphorylation [28]. The latest fascinating addition being serotonylation and its involvement in permissive expression [29]. Post-translational modifications of histones regulate the shift from a tighter chromatin (heterochromatin) to a looser chromatin (euchromatin) state, or vice versa [30]. The heterochromatin state encapsulates either constitutive heterochromatin, with associated H3K9me3 and high levels of DNA methylation found in pericentromeric regions and telomeres; or facultative heterochromatin, with high H3K27me3 in nonrepetitive regions and genes silenced via histone deacetylation [31]. Histone tail modifications thus result in either transcriptional activation or repression through the action of different modification writing and erasing enzymes (see Figure 1) [30]. For instance, the mechanism of acetylation is regulated by the action of histone acetyltransferases (HATs), which catalyze the addition of acetyl groups using acetyl-coA as a coenzyme, and histone deacetylases (HDAC) that catalyze their removal. Lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) are critical and well-studied enzymes with acknowledged roles in pathology [32]. In general, histone acetylation influences or is correlated with transcription, whereas deacetylation is associated with an inactive chromatin state [33]. At both promoters and enhancers, classically delineated by H3K4me3 and H3K4me1 modifications, respectively, the co-occurrence of acetylation in the form of H3K27ac indicates activity at these regulatory regions, compared to only a likely permissive/poised state without [34].

1.3 Non-coding RNA

An extremely high proportion (>~98%) of the transcribed human genome does not encode proteins but instead numerous non-coding RNAs (ncRNAs) [35], which are divided into two classes: regulatory and structural. According to their length, regulatory ncRNAs can be further categorized into two major groups: (i) small ncRNAs (<200 nucleotides) that include microRNA (miRNA) and PIWI-interacting RNAS (piRNA), and (ii) long ncRNAs, which are longer than 0.2 kb [36]. ncRNAs can influence the expression of other genes (e.g. CHD2 by the lncRNA *Chaserr* [37]) and are involved in chromatin regulation and remodelling through the interaction with transcription factors and epigenetic regulators to adjust their retention or catalytic activity [38]. They act with both methyl- or acetyl- writing and erasing

188 enzymes, resulting in a change in chromatin conformation and gene activity [39].
189 lncRNAs can function as chromatin remodellers and can act as scaffolds for
190 additional proteins or protein complexes (e.g. lncRNA *KHPS1* activating a poised
191 enhancer)[36,40–42]. Moreover, miRNAs are found to impact epigenetic
192 processes by directly inhibiting the role of some enzymes related to DNMTs,
193 histone modifications and chromatin remodelling or by changing the availability
194 of substrates required for these enzymatic reactions. Hence, a disruption in the
195 miRNA-epigenetic regulatory circuitry may result in the impairment of normal
196 chromatin function, leading to various diseases [33].
197

198 **2. Epigenetics as a biomarker of cardiometabolic disease**
199 Epigenetic biomarkers, specifically DNA modifications, are highly attractive for
200 clinical practice because of their long-term stability [43]. They are molecular
201 markers with the ability to accurately quantitate environmental, lifestyle and
202 endogenous contributors to disease (*e.g*. pollution, smoking and inflammation
203 *etc.*) [4,44–46]. Cardiometabolic disease is associated with many risk factors that
204 can be detected by epigenetic biomarkers, as well as concurrently observable
205 epigenetic network modulations driving its pathogenesis.
206

207 ### 2.1 Ageing-associated changes
208 Ageing is an obvious but major risk factor for cardiometabolic-related phenotypes
209 [47] due to many interrelated pathophysiological mechanisms [48]. It constitutes
210 progressive damage of tissue and reduced organ function over a period of time.
211 Oxidative stress and cellular senescence are involved in ageing and many acute
212 and chronic pathological processes, including cardiovascular, cardiometabolic,
213 acute and chronic kidney diseases [49]. Epigenetic alterations are one of the
214 hallmarks of ageing [47]. As cells age, we observe widespread changes in
215 transcription factor binding, heterochromatin formation, DNA methylation and
216 the histone modification landscape [50]. Several specific CpG sites that undergo
217 consistent directional age-related methylation changes have been determined and
218 enabled the construct of age predictors or DNA methylation 'clocks' [51]. These
219 clocks have been shown to capture 'biological' aspects of ageing, which are good

predictors of cardiovascular disease (CVD) and all-cause mortality [52–54]. One gene involved in ageing and is modulated through epigenetic changes is *SIRT1*. It is an important gatekeeper against inflammation, oxidative stress as well as deterioration of cardiovascular structure and function and is found to be downregulated in ageing [55]. Due to recent technological advances there is significant interest in the analysis of the ageing-related epigenomic changes, as these may bring novel functional insights to ageing-related disease mechanisms, including cardiometabolic disorders [54,56,57].

Another fascinating insight into ageing, with ramifications for both epigenomics and CVD, is age-related clonal haematopoiesis (ARCH) [58]. With age, somatic mutations accumulate in the DNA of hematopoietic stem cells, and those conferring a competitive advantage to the mutated cell result in its clonal expansion [59]. Clonal haematopoiesis has been associated with haematological malignancy, but additionally recent findings have connected this process with increased cardiovascular risk, with a potential role as a driver of atherosclerosis [59]. Therefore, clonal hematopoiesis is identified as being a possible novel biomarker of vascular ageing [47,59] and is also associated with epigenetic measures of biological age [60]. More than 70% of cases of ARCH are a result of mutations in the genes coding for *DNMT3A*, *TET2* and *ASXL1*, which modulate DNA and histone methylation and thus lead to distinct clonal epigenome-wide changes [61]. Causal evidence has also come from a mouse model, where TET2-deficient macrophages increased NLRP3-mediated inflammasome IL-1beta secretion and, thus, accelerated atherosclerotic plaque growth [62].

2.2 Hypertension

Modifications in the epigenome are identified in essential hypertension [63,64]. Many genes related to pathways and biological processes involved in hypertension are regulated by epigenetic changes. One example is *HSD11B2*, a gene responsible for catalysing the conversion of cortisol to cortisone, with promoter DNA hypermethylation resulting in abnormal levels of cortisol- and cortisone-active metabolites and consequently in the onset of essential hypertension [63,65]. Moreover, there is evidence that endothelial nitric oxide synthase (eNOS) activity is downregulated in CVD by cell-specific histone

modifications [66]. This enzyme is responsible for NO production in the vascular endothelium and plays an essential role in the regulation of vascular tone [67,68]. A recent epigenome-wide study (EWAS) in peripheral blood for hypertension identified 7 significant CpGs in a European ancestry cohort for Diastolic blood pressure but only 1 (cg07598370 near *OR5AP2*) in a trans-ancestry analysis including a South Asian ancestry group [69]. However, differentially methylated region (DMR) analysis in the trans-ancestry cohort identified numerous Systolic (n=387) and Diastolic (n=237) DMRs. Of these DMRs, 12 were in common between both traits, including overlap with *TFAP2D* and *HLX* previously genetically implicated with hypertension [70,71].

2.3 Obesity and Type 2 Diabetes

Obesity and type 2 diabetes (T2D) are also detectable in changes to the epigenome. Costantino *et al*. determined that the mitochondrial adaptor $p66^{Shc}$ is upregulated in visceral fat arteries of obese patients and is associated with endothelial dysfunction, oxidative stress and insulin resistance [72]. A group of chromatin remodelers were found through unbiased gene profiling and chromatin immunoprecipitation to regulate $p66^{Shc}$ transcription by inducing demethylation and H3K9 acetylation [72]. Interestingly, intensive glycemic control failed to revert $p66^{Shc}$ -related epigenetic modifications in peripheral blood monocytes of T2D individuals [73]. T2D is a heterogenous disorder associated with obesity, inflammation and insulin resistance [74]. Furthermore, in T2D, pancreatic β-cells, whose dysfunction is a dominant contribution to this disease's development [75], undergo modifications in gene expression. DNA methylation has a significant role in the development of the β-cell [76] but also in the age-related progression of T2D [77]. The importance of the regulation of islet epigenetics by DNA methylation is demonstrated in that combined knock-out of Dnmt1 and the homeobox gene Aristaless-related homeobox (Arx) convert adult islets α-cells into functional β-cells [78]. Hyperglycaemia has been implicated in the β-cell dysfunction and altered celluar identity that occurs in T2D [79]. Intergrated β-cell epigenomic analysis also revealed weakening of β-cell specific Polycomb Repressive Complex 2 (PRC2) bivalent domains leads to ectopic gene activation in T2D patients [74]. Loss of transcription integrity of lineage-defining genes was also observed. These

polycomb-dependent changes were proposed as amenable epigenomic therapeutic targets to stabilise β-cell identity with also the potential for directing α- to β-cell conversion [80]. *PRMT1* methylates arginine-3 of histone H4 (H4R3me2a), enables p300 to acetylate further H4 residues, and is also found to be required to maintain mature β-cell state [81]. Recent evidence also points to the critical role of the lnc-RNA, lncRNA-*PAX6-AS1*, with high glucose states driving increased expression of this repressor of the pancreatic β-cell identity and functional transcription factor Pax6 [82].

Volkov et al., using whole-genome bisulfite sequencing, performed the first comprehensive analysis of the DNA methylome of a T2D case-control cohort in pancreatic islets [83]. A very large number of DMRs (n=25,820) were found in islets from T2D patients compared to controls. However, the key islet transcription factor, *PDX1,* was an outlier overlapping with seven DMRs covering >2.5kb, which included 105 CpGs, and this result was also independently replicated. The total DMR set showed enriched overlap with binding sites for islet-specific transcription factors. When integrating the DMR analysis with pancreatic islet RNA-seq data, *SOCS2*, *PARK2*, *PID1* and *NR4A3* showed changes in both DNA methylation and expression. Functional studies of these genes in rat β-cells revealed that the expression changes impaired glucose-stimulated insulin secretion [84]. Furthermore, islet PDX-1, which has a reduced expression in T2D patients, showed increased methylation when exposed to glucose [85,86]. PDX-1 is also regulated through histone methylation by the action of Set7/9, a lysine methyltransferase (the mouse ortholog of human SETD7; KMT7) [87].

An epigenetic role in the complications of diabetes, such as nephropathy has also been explored [88,89]. Khamis *et al*. recently identified an intriguing epigenetic connection between lipid-lowering statins and the insulin sensitivity role of mature adipocytes [90]. Statins are known to increase T2D risk [91] and their experimental administration led to promoter hypomethylation and increased expression of the epigenomic-erasor *HDAC9*, which in turn reduced the activity of crucial adipogenic genes, such as *ABCG1* [90]. The impact on adipocyte maturation subsequently increased insulin resistance. This mechanistic insight will need to be carefully appraised, as both HDAC9 and ABCG1 are considered potential therapeutic targets for metabolic diseases.

From three meta-analysis EWAS performed in peripheral blood for obesity [92,93], ten significant CpGs were consistently identified [94]. None have any biological evidence of being causative in obesity, but instead are consequential to the obese state, such as changes driven by hyperlipidaemia, hyperglycaemia and inflammation. In line with this, a meta-analysis of EWAS in only children and adolescents (n=4,133 from 23 studies) found minimal DNA methylation association with childhood and adolescent BMI [95]. However, with advancing age there was an increasing overlap with known associations observed with adult BMI. Such findings further support that differences in blood DNA methylation are a consequence rather than a cause of obesity.

2.4 Dyslipidemia

Epigenomic modifications have been implicated with a role in inter-individual postprandial lipemia (PPL) variability. An epigenome-wide association study for PPL in isolated CD4+ T cells, from 979 adults from the GOLDN study challenged with a high-fat meal, identified eight significant DNA methylation sites involving five genes (*CPT1A*, *LPP*, *APOA5*, *SREBF1* and *ABCG1*) [96]. The increased methylation at *LPP*, *APOA5*, *ABCG1* and *SREBF1* and the decreased methylation at *CPT1A* correlated with the increase of triglyceride-PPL response [96]. With the same cohort, another study revealed *CPT1A* methylation being associated with fasting very-low LDL-cholesterol and triglyceride [97]. *CPT1A* has been previously pinpointed in multiple cardiometabolic-related EWAS [94]. A landmark study from Dekkers *et al*. [98] determined, using multivariable Mendelian randomization, that DNA methylation change is induced by triglycerides at three CpGs, by HDL cholesterol at two CpGs, and by LDL cholesterol at one CpG. These six CpGs were found to be associated with the expression of key regulators of lipid metabolism, which include *CPT1A* and *SREBF1*, *ABCG1*, and *DHCR24* for triglycerides, HDL cholesterol, and LDL cholesterol, respectively.

A recent EWAS of metabolic syndrome and its components was conducted on 1,887 individuals of European ancestry [99]. The study implicated cg08309687, a locus that was previously associated with T2D, with lipid metabolism. Another DNA methylation locus, cg17901584, was found to be connected with HDL levels.

Also, the study concluded that cg19693031, within the 3'UTR of *TXNIP*, possesses multiple intersecting lines of evidence for it to be a potential central epigenetic hub locus linking separate metabolic syndrome components. A further EWAS in peripheral leukocyte DNA of 226 metabolic measurements determined by nuclear magnetic resonance (NMR) spectroscopy identified 161 robustly replicated associations for 57 unique metabolic measures at 16 CpG sites (discovery n= 1662, replication n = 3752) [100]. All these CpGs had been previously associated via biochemical and clinical measures with metabolic components.

In regard to non-coding RNA, two lncRNAs have been determined as powerful regulators of lipid levels as well as atherosclerosis: LeXis, which is expressed in the liver and controls genes involved in cholesterol biosynthesis, and MeXis, which is expressed in macrophages and controls genes involved in cholesterol efflux [101,102].

2.5 Atherosclerosis

The pathogenesis of atherosclerosis involves variations in epigenetic modifications and gene expression in a cell type- and stage- specific manner [103]. Significant changes in DNA methylation occur in the atheroscelerotic plaque [104] with early studies on humans and mice noting global and focal DNA hypermethylation [105,106]. Using bisulfite sequencing of healthy and atherosclerotic human aortas, a positive relationship between DNA methylation and atherosclerotic lesion grade was determined [107]. A DNA methylation array analysis comparing aortic healthy versus normal identified >1,800 plaque-associated CpGs and validated 16 by pyrosequencing, including promoter CpGs with inversely associated transcription levels for *HOXA6* and *MIR23b* [105]. A more recent 850k DNA methylation array analysis identified 2,679 CpGs reaching genome-wide significance between paired atheromatous plaque lesions and corresponding plaque-free aortic intima tissue in 128 post-mortem specimens from 64 Japanese patients [108].

In vitro studies have suggested that inflammatory signaling pathways can regulate DNA methylation [103]. For instance, proinflammatory stimuli, such as oxidized LDL (oxLDL), resulted in DNMT1 upregulation and caused the DNA methylation of the promoter of the gene that encodes Krüppel-like factor 2 (*KLF2*) [109]. KLFs

383 belong to a group of anti-inflammatory transcription factors, thus *KLF2*
384 suppression increases proatherogenic endothelial inflammation [109].

385 Post-translation histone tail methylation and acetylation modifications occur in the
386 development of atherosclerosis as well as its progression to atheroma involving a
387 number of different cell types, including monocytes, macrophages, vascular smooth
388 muscle cells and endothelial cells [110]. A distinct subset of CD4+ T cells with
389 expression markers of T cell exhaustion are observed in the atherosclerotic lesions
390 [111]. Macrophages become activated and with uptake of lipid particles differentiate
391 into foam cells, becoming further dysregulated in the plaque [112].

392 Several lncRNAs have been identified as key players in vascular diseases [103].
393 One example is *ANRIL*, which is NF-kB inducible [113] and promotes human
394 vascular smooth muscle cell (vSMC) proliferation [114,115]. It also upregulates
395 the expression of IL6 and IL8 through interaction and recruitment of the
396 transcription factor YY1 [113]. Additionally, long intergenic non-coding RNA p21
397 (lincRNA-p21) is implicated in atherosclerosis [36]. Its expression is
398 downregulated in atherosclerotic plaques of $Apoe^{-/-}$ mice and human coronary
399 arteries [116]. Moreover, the knockdown of this lncRNA stimulated the expansion
400 of the neointima in a mouse model of carotid artery vascular injury [116].

401

402 **3. Potential use of epigenome-modifying drugs in cardiometabolic disease**
403

404 ### 3.1 Success with therapeutic manipulation of the epigenome in cancer

405 Since the clear recognition of a global loss of DNA methylation in cancer cells in
406 1983 [117], epigenetic drug discovery efforts have investigated oncogenic
407 pathways [118]. Epigenome modulation is a feature of almost all human cancers;
408 different mutations in chromatin-controlling genes are common, some of which
409 are drivers for tumour initiation, while others may influence immune invasion,
410 metastasis or even drug resistance [119]. Therefore, epi-drugs in cancer are
411 targeted at reversing these changes and reactivating tumour suppressor genes
412 [120]. These therapies tested in clinical trials can be divided into two major
413 groups: i) broad 'reprogrammers' and ii) targeted therapies. Broad

reprogrammers cause genome-wide modifications in the epigenome, and include DNA methyltransferase inhibitors (DNMTi), histone deacetylase inhibitors (HDACi), and inhibitors of the bromodomain and the extra-terminal motif proteins (BETi). These were the first-generation modulators to be permitted for clinic use. Targeted therapies, on the other hand, treat specific genetic mutations in the epigenetic pathways [121–123], or in the future, epigenetically modify a specific regulatory locus.

Although many drugs are in development, presently nine epigenetic agents have been approved by the FDA in the US for the standard treatment of haematological cancers and solid tumours [13]. These are currently from the writer and eraser category of epigenomic regulators and include DNMTi, HDACi, IDHi (Isocitrate dehydrogenase inhibitors that block IDH mutant inhibition of TET2 and lysine demethylation) and EZH2i (inhibition of the EZH2 involved in H3K27 methylation and the enzymatic component of the Polycomb Repressive Complex 2) [13]. Interestingly, most of the work on multidrug combinations is currently focused on the synergistic role of epi-drugs as potentiators to increase sensitivity to other anticancer therapies, such as immunotherapy in patients with cancer [124–126]. Both HDACi and DNMTi have shown an effective immunomodulatory activity on host immune cells and tumour cells.

3.2 Toward epigenetic therapies for cardiometabolic disease

Altered epigenetic regulation has been related to the development and progression of cardiometabolic disease and the modulation of the biological processes underlying it. The six currently approved epigenomic eraser inhibitors, which are all HDACis, are: Vorinostat, Belinostat, Panobinostat and Romidepsin, plus repurposed compounds now recogised to affect this pathway; the diet supplement Sodium Butyrate [127] and the anticonvulsant and affective disorder treatment, Valproic acid (see Table 1). Therefore, after their success as epigenomic modulators in cancer therapy, HDACis experimental benefits have been explored across a range of diseases, including cardiovascular, neurodegenerative, and inflammatory diseases (e.g. Atherosclerosis [32], long-term memory [128], Systemic Lupus Erythematosus (SLE) [129]). This has

subsequently led to several clinical trials now taking place to study the use of epigenetic drugs in targeting conditions related to cardiometabolic disease. Supportive evidence for HDACis for the treatment of metabolic and cardiovascular diseases [130], also includes improved endothelial dysfunction and diabetic nephropathy, and reduced ischemia/reperfusion myocardial injury [131,132]. Humans possess 18 HDACs, which can be categorized into four major classes according to their homology to yeast (class I, II, & IV possessing conserved deacetylase domains differing by specific cofactor dependence, and class III sirtuin proteins) [133]. Whilst there is significant experimental evidence for cardiovascular benefits of HDACis [134], the inhibition of the distinctive classes of HDAC differ in their action. Inhibitors of Class II and class III HDACs have protective roles, not only in heart injury but also in vessel injury, whereas those acting on class I HDACs protect against vessel damage [135], but can led to cardiac arrhythmias, atherosclerosis and vessel calcification [32].

Administration of Trichostatin A (TSA), a selective class I & II HDAC inhibitor, in a mouse model of acute myocardial infarction reduced inflammation by downregulating TNF-α expression and stimulated angiogenesis through AKt-1 phosphorylation, leading to ventricular remodeling reduction [136]. Vorinostat (SAHA; suberanilohydroxamic acid), which is approved for the treatment of Cutaneous T-Cell Lymphoma (CTCL), has also gained interest due to its ability to reduce inflammation and oxidative stress [137,138]. Oral administration for 18 weeks in diabetic mice, resulted in the reduction of albuminuria, collagen deposition and oxidative-nitrosative stress by inhibiting eNOS coupling [139]. In addition, in a rabbit model of myocardial infarction provoked by ischemia followed by reperfusion, SAHA was shown to reduce myocardial infarct size and partially improve systolic function [132]. This was by inducing cell death and cardiomyocyte autophagy, a mechanism implicated across a range of disorders [140]. However, the molecular mechanisms underlying autophagy in cardiac muscle cells remain unknown [132]. Similarly, the HDACi Sodium Butyrate was found to lessen the inflammatory response in atherosclerosis and myocardial infarction by suppressing the inflammatory molecules TNF-α, IL-6, ICAM-1 and VCAM-1 and driving cardiomyocyte autophagy [132,141]. Also, this compound has shown therapeutic effects in diet-induced obesity by increasing mitochondrial

480 oxidative phosphorylation and activating adiponectin-mediated pathways as well
481 as the ability to reduce low-grade inflammation, vascular disease, and protect
482 against cardiac dysfunction in T2D and obesity [127,141,142].
483 Resveratrol, a polyphenol that is present in red wine, is recognized to activate the
484 histone deacetylase SIRT1, and has been implicated in improving endothelial
485 function, insulin sensitivity and myocardial dysfunction in obese and T2D patients
486 [143]. Interestingly, resveratrol and its derivatives may also play a role as DNMT3
487 inhibitors [138,144]. However, its rapid metabolism and poor bioavailability are
488 acknowledged weaknesses [145]. Therefore, the benefit of this compound
489 requires further clinical studies to be robustly assessed; it exhibits a broad range
490 of cardioprotective, vasculoprotective and atheroprotective-related activities,
491 suggesting its therapeutic potential for the treatment, or prevention, of
492 cardiometabolic disease [138]. The anti-diabetic medications metformin and
493 glucagon-like peptide (GLP-1) analogues are also modulators of SIRT1 activity,
494 thus modifying histone acetylation and transcription of genes involved in insulin
495 signaling and pancreatic β-cell homeostasis [39].
496 A promising future for DNA methylation modifier clinical therapies is suggested
497 as aberrations in DNA methylation patterns, as well as being biomarkers of
498 disease, can also potentially be drivers of cardiovascular and metabolic disorders.
499 Increased DNA methylation was observed in some atheroprotective genes
500 including those encoding estrogen receptors (ER)-α and -β (ESR1 and ESR2,
501 respectively) in human coronary atherosclerotic tissues and plaque regions of
502 ascending aorta [146]. Hypermethylation of ESR1 and ESR2 was also detected in
503 *in vitro* senescing smooth muscle cells and endothelial cells [147]. Such findings
504 imply that epigenetic modifications in both ESR1 and 2 can drive vascular ageing
505 and atherosclerosis [147]. The DNMTi 5-aza-2-deoxycytidine (5-AZA), which is
506 now a well-established anti-cancer therapy, administered in addition to TSA,
507 restored the expression of ER genes in normal smooth muscle cells and
508 endothelial cells, showing low toxicity in these cells [138,148]. Therefore, the
509 combination of epigenetic therapy with hormone replacement therapy has been
510 proposed as potentially beneficial for the prevention and/or treatment of CVD
511 [149]. Experimental intraperitoneal injections of 5-AZA in $Ldlr^{-/-}$and $Apoe^{-/-}$

mouse models was observed to cause a reduction in the atherosclerotic lesion burden [150,151].

Different natural compounds can also affect relevant epigenetic networks and consequently prevent cardiometabolic features. For instance, Curcumin, a naturally occurring molecule that gives turmeric its yellow color, modulates the activity of DNMT1, HATs (including p300) and HDACs, and has the potential to prevent several diabetes-related outcomes as well as recognized cardiovascular protective effects [137,138,152]. Administration of Curcumin in rats averted the development of hypertension-induced heart failure by inhibiting GATA4 transcription, which is a hypertrophy-responsive transcription factor [153]. The atheroprotective action includes enhancing cholesterol efflux in human and mouse macrophages *in vitro*, with an anti-inflammatory effect acting through the downregulation of NF-kB activity [154–156].

Similarly, dietary supplementation of folate, a crucial vitamin responsible for the production of SAM, has the ability to revert aberrations in chromatin in different cardiometabolic states. Folate-enriched diet reduced endothelial dysfunction and hypertension as well as increasing the bioavailability of nitric oxide [157]. In addition, a study demonstrated that folic acid supplementation prevents atherosclerosis by investigating its atheroprotective role in high-fat diet-fed *Apoe* knockout mice and in oxidized low-density lipoprotein-treated human umbilical vein endothelial cells [158]. An increase in folic acid levels elevates DNA methyltransferase activity and expression, and thus modifies the expression of atherosclerosis-related genes [158]. Several other compounds like apicidin, valproic acid and peroxisome proliferator-activated receptor gamma agonists (PPARγ-agonists) also have the ability to revert chromatin alterations in cardiometabolic conditions (see Table 1) [39,137].

The BET protein family belongs to the class of epigenomic readers [13]. They modulate gene transcription through their interaction with acetylated histone residues, and they have been implicated in different pathological conditions, including inflammation [137]. The BET inhibitor Apabetalone (RVX 208) is able to regulate reverse cholesterol transport, coagulation, vascular inflammation and complement activation in cultured primary human hepatocytes [159]. Moreover,

a pooled analysis of phase II clinical trials for this BETi demonstrated a significant reduction of major adverse cardiovascular events (MACE) [160]. A phase III clinical trial on T2D individuals with recent acute coronary syndrome revealed promising outcomes related to specific secondary endpoints like cardiovascular death or nonfatal myocardial infarction and heart failure. However, this data could not clearly establish a reduction in MACE [161]. Although, a short-term analysis of established coronary artery disease patients showed benefit with respect to cardiovascular events compared to placebo [162]. Additionally, a recent study assessed the effect of Apabetalone on *ex vivo* inflammatory responses of monocytes from patients with T2D and CVD [163]. They demonstrated that Apabetalone treatment suppresses this pro-inflammatory phenotype, which further supports the potential role of BETi as a therapeutic agent for high risk T2D and CVD patients.

3.3 Challenges and future directions

Whilst our understanding of the complexity of the epigenome is still limited, pharmacological targeting of epigenetic networks will remain challenging [4]. The variability in cellular composition can confound or dilute biomarker studies due to the cell-type specificity of epigenetic factors, particularly for rare cell subtype signatures. Moreover, in most cases, detailed functional evaluation is required to distinguish whether the epigenetic modification is a consequence of disease and possibly helpful for diagnosis, patient stratification or response prediction, or a cause that could be a suitable therapeutic target [4]. Another issue to be addressed when considering implementing epigenetic therapy is the lack of locus specificity. Genome-wide epi-drugs can disrupt epigenetic marks indiscriminately, which may modulate the expression of off-target genome sequences. This can be accompanied with substantial side effects [164,165]. The CRISPR-Cas9 editing system has been modified to perform locus-specific epigenetic editing [166], including DNA methylases [167], demethylases, and histone modifiers, including acetylators, deacetylators and demethylators [168]. Using dCas9 fused to different epigenetic modifiers and transcriptional regulatory domains, or using scaffold RNAs recruited with effector proteins, CRISPR-Cas9 system-mediated site-specific

transcriptional and epigenetic modulations can be achieved [168–170]. Although targeting efficiency and intracellular delivery are still considerations [171], CRISPR-Cas9 is a very promising tool for the precise modulation of the cardiometabolic disease epigenome [172]. Illustrating the capability of epigenomic editing , a study succeeded in stimulating human β-cell proliferation even using the older TALE targeting methodology coupled with the TET1 enzyme [173]. In the imprinting disorder Beckwith-Wiedemann syndrome (BWS), β-cell hyperproliferation is driven by hypomethylation of the materal imprinting control region 2 (ICR2) of the *CDKN1C* gene (expressing the cell-cycle inhibitor p57). In this study, the epigenomic targeting of the TET1 demethylating enzyme to this *CDKN1C* ICR2, produced a similar increase in β-cell mass. The CRISPR/CAS9-based genetic deletion of enhancers induced by the common hypertensive therapeutic target angiotensin II (AngII), which also promotes atherosclerosis by growth and pro-inflammatory pathways in vSMCs, blocks this AngII-induced pathology [174]. These knocked-out enhancers are clear cardiovascular targets for more naunced future therapeutic epigenomic modulation.

Conclusions

Clinical epigenetics is still in a developmental phase. Despite its infancy, a few epi-drugs have been approved and implemented in clinical use, and a variety of epigenetic biomarkers for disease diagnosis, prognosis or response to therapy are now available or are in development. Currently, oncology is the main focus for epi-drugs, but an increasing number of trials suggest their potential for use in many other non-malignant conditions in the very near future. In this review, we have shed light on the strong prospects of these epigenetic modulators in alleviating the progression of cardiometabolic disease. Furthermore, a deeper understanding of the epigenetic landscape in disease enabled by advancing technologies, including single cell analyses, may reveal additional novel targets for the prevention and treatment of cardiometabolic-related disorders. Epigenetic drugs represent true genomic therapeutics, and the development of a new era of personalized genomic medicine using predictive biomarkers or personalized therapy will undoubtedly incorporate the epigenome as a vital player.

608

609 **Conflicts of Interest**

610 The authors have no conflicts of interest to declare.

611 **Table 1**

612

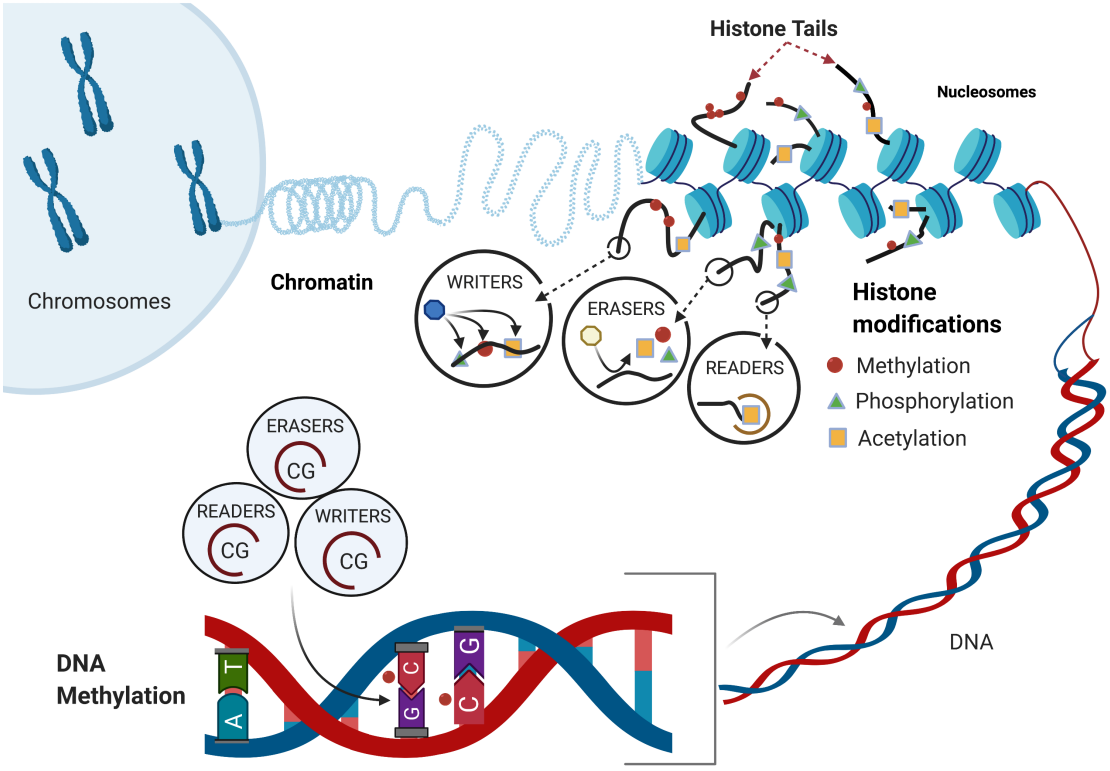
Compound	Epigenetic Mechanism	Models Tested	Effect
Apabetalone	BET inhibition	Human, Mouse	Modulates reverse cholesterol transport, coagulation, vascular inflammation and complement activation [159] and reduces cardiovascular events in patients with coronary artery disease [175].
Apicidin	HDAC inhibition	Human cells, Mouse	Reduces myocardial hypertrophy after 1-week pressure overload prompted by thoracic aortic constriction [176].
Curcumin	HAT inhibition	Rat	Inhibits cardiac hypertrophy and preserves cardiac/endothelial function [177].
Folate	DNA and histone methylation	Human cells, Mouse	Influences SAM production, modifies the expression of atherosclerosis-related genes and prevents atherosclerosis [158].
GLP1 analogue	SIRT1 activation	Mouse	Reduces blood pressure, vascular inflammation, and oxidative stress and prevents uncoupling of eNOS [178].
Metformin	SIRT1 activation	Human	Protects vascular endothelial cells from cellular stress, downgrades signs of cellular ageing and production of reactive oxygen species [179].
PPARγ agonists	HAT/HDAC recruitment	Human	Improve vascular function in T2D patients [180].

Resveratrol	SIRT1 activation	Human, Mouse	Improves endothelial function, cardiac dysfunction and insulin sensitivity in obesity and T2D [143].
Sodium Butyrate	HDAC inhibition	Human cells, Rat	Suppresses inflammatory cytokines in experimental models of myocardial infarction and atherosclerosis [141].
Trichostatin A	HDAC inhibition	Human cells, Mouse	Promotes angiogenic response and cardiomyocyte survival and prevents ischemia-induced left ventricular remodelling [136].
Valproic Acid	HDAC inhibition	Rat	Attenuates hypertrophic and hypertensive responses through modulation of ROS-generating and pro-inflammatory pathways [181].
Vorinostat	HDAC inhibition	Mouse, Rabbit	Promotes the autophagic flux, prevents uncoupling of eNOS and activation of NF-kB and reduces oxidative stress [132,139,182].

613

Table 1 Epigenetic drugs assessed for treatment of cardiometabolic disease, in alphabetic order [39,137]. BET: Bromodomain and the Extra-Terminal motif; eNOS: Endothelial Nitric Oxide Synthase; GLP1: Glucagon-Like Peptide 1; HAT: Histone acetyltransferase; HDAC: Histone Deacetylase; NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma; ROS: Reactive Oxygen Species; SAM: S-adenosylmethionine; SIRT1: Sirtuin 1, a NAD^+-dependent Histone Deacetylase; & T2D: Type 2 Diabetes. 614

615 **Figure 1**



616

617 **Figure Legend**

618 Figure 1. The Epigenome

619 The epigenome comprises of chromatin, a nucleoprotein-complex composed of
620 DNA and histone proteins, which make up the chromosome in eukaryotic cells. It
621 interacts with DNA modifications, post-translational histone tail modifications,
622 and non-coding RNA molecules. Figure adapted from Bates [13] and Keating *et al.*
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