

Epigenetic mechanisms of vascular dysfunction in obesity and type 2 diabetes

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Summary

Over the last decade, several studies have shown that epigenetic modifications – defined as changes to the genome that do not alter the DNA sequence – may affect the expression of genes involved in inflammation, oxidative stress and angiogenesis. These alterations were recently shown to promote cardiovascular disease in the setting of obesity and type 2 diabetes. Most importantly, epigenetic modifications acquired during life may be transmitted to the next generations and may contribute to pathological cardiovascular traits already present during childhood. In the context of diabetes and obesity, high blood glucose levels and insulin resistance lead to modifications of the epigenome (DNA methylation, histone marks, noncoding RNAs), which may account for a persistent impairment of vascular and cardiac function, even after correction of underlying risk factors (intensive glycaemic control, weight loss). Hence, a careful assessment of the “epigenetic landscape” in cardiometabolic patients may contribute to the estimation of global cardiovascular risk, thus providing the tools for a personalised therapeutic approach. The present review describes the emerging role of epigenetics in the pathogenesis of cardiovascular complications in obesity and type 2 diabetes.

Keywords: epigenetics, diabetes, obesity, miRNA

Obesity and diabetes worldwide

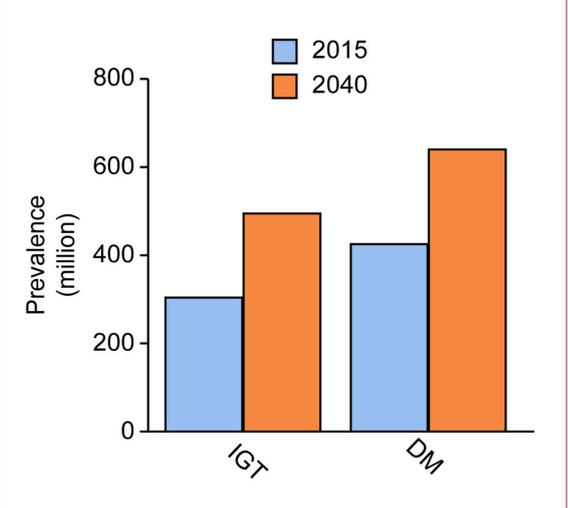
Data from the International Diabetes Federation (IDF) indicate that 316 million people are currently affected by impaired glucose tolerance (IGT) and this number is expected to reach 470 million by the year 2035 [1]. A disproportionate increase in the prevalence of obesity implies a dramatic increase of people affected by type 2 diabetes (T2D). Indeed, IDF forecasts anticipate that, in 20 years, 640 million people will be affected by diabetes worldwide (fig. 1). This equates to approximately three new cases every 10 seconds, or almost 10 million per year [1]. Most importantly, almost 50% of affected people are unaware of their condition and do not receive treatment. The progression from obesity to T2D may take several years to occur and involves various cellular mechanisms including alterations of insulin sensitivity, glucose transport and pancreatic beta cell dysfunction, as well as alteration of genes regulating oxidative stress and inflammation [2]. During this transi-

tion, a progressive deterioration of cardiovascular function is also observed. As yet, the fine molecular alterations responsible for the impairment of vascular function across the spectrum of obesity and T2D remain to be elucidated.

Epigenetic mechanisms

Over the last decade, a growing number of studies have contributed to the unveiling of the contribution of epigenetics in the pathophysiology of diabetes-related cardiovascular disease [3]. Epigenetic regulation of gene expression mainly affects DNA activity without altering its sequence. In genetically identical cells, epigenetic modifications may differentially orchestrate transcriptional programmes, thus leading to different phenotypic traits [4]. Epigenetic diversity among individuals also contributes to the explanation of monozygotic twin discordance rates for common diseases, such as asthma [4, 5]. Epigenetic mechanisms are mainly induced by environmental stimuli and can be divided into three main categories: (i) DNA methylation, (ii) histone modifications, and (iii) noncoding RNAs (ncRNAs) (fig. 2) [7]. Methylation of DNA and posttranslational modifications of histones take place at the level of chromatin, whereas ncRNAs mainly act at the posttranslational and posttranscriptional levels. DNA

Figure 1: Worldwide prevalence of impaired glucose tolerance and diabetes in 2015 and 2040. Adapted from International Diabetes Federation Atlas, 7th edition.



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and histones are packaged into nucleosomes. The latter are composed of a 147bp DNA strand wrapped in a superhelical way around a core of histone octamer (two H2A-H2B dimers and a H3₂-H4₂ tetramer). Chromatin may acquire two different functional states in cells: it can be packed in a structure that is inaccessible for transcription (heterochromatin) or it may assume a looser, uncondensed structure, which enables gene transcription (euchromatin).

DNA methylation

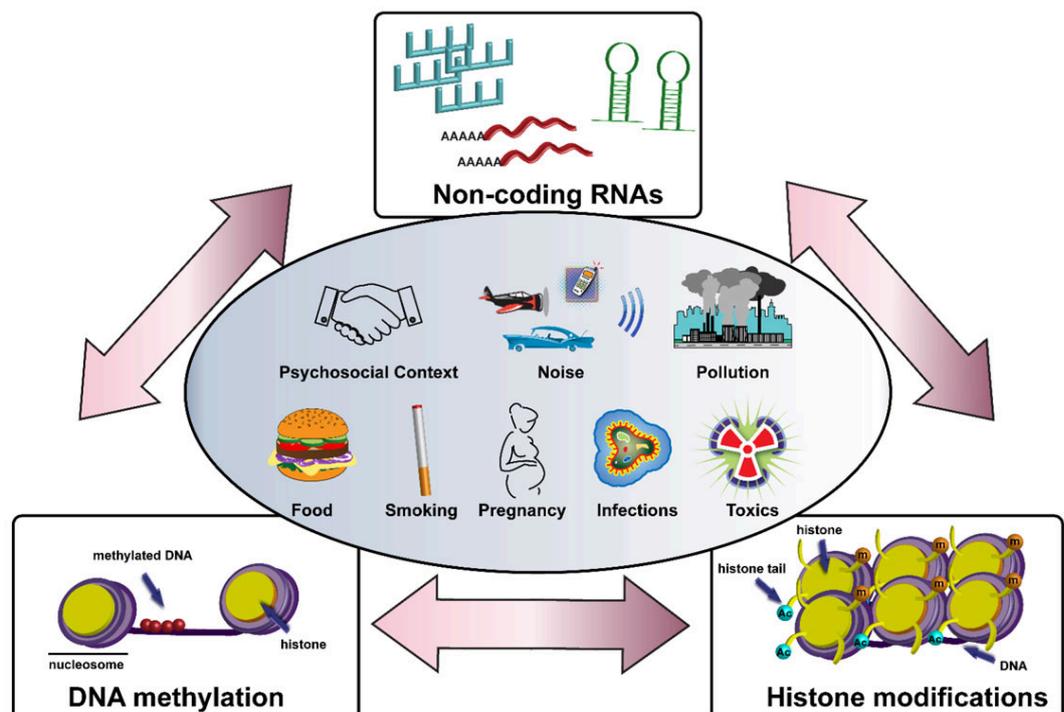
DNA methylation is a covalent modification predominantly occurring at cytosine residues that are followed by guanine (CpG dinucleotides) to form 5-methylcytosine [8]. Methylation of CpG regions mainly takes place at the level of gene promoters, where it plays a pivotal role in regulating gene activity and transcription. Promoter methylation hampers gene expression mainly via two mechanisms: (i) by preventing the recruitment of transcription factors, or (ii) by fostering transcriptional silencing [9, 10]. X-chromosome inactivation represents an example of how epigenetic silencing by DNA methylation may control gene transcription [8, 11]. Different families of enzymes, known as methyltransferases (DNMTs), are involved in the regulation of DNA methylation: DNMT1 is responsible for the maintenance of methylation, whereas DNMT3a and DNMT3b are implicated into *de novo* methylation of DNA [9]. Methylation of DNA is a dynamic and reversible process governed by methyl-writing and -erasing enzymes. Reduced DNMT1 activity or increased expression of ten-eleven translocation enzymes (TETs), a family of methyl-

erasing enzymes, are fine regulators of promoter methylation and gene expression [9, 12].

Histone modifications

Several posttranslational modifications at the level of histones (acetylation, methylation, ubiquitination, phosphorylation, sumoylation, citrullination, and ADP-ribosylation) contribute to the regulation of chromatin architecture [7]. These plastic changes repress or activate gene transcription by modifying the chromatin state (euchromatin or heterochromatin). In other words, modifications of histone tails modify the interaction between histones and DNA thus affecting chromatin accessibility in specific regions (depending on the type of modification and on the position of the modified residue). For example, acetylation of lysine residues modifies the binding strength of DNA to the histone by neutralising the negative charge of the ε-amino group of lysine. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are families of enzymes regulating histone acetylation [13]. Whereas acetylation is generally associated with chromatin accessibility and enhanced gene expression, the same does not apply to histone methylation, which can either enhance or repress gene transcription depending on which amino residue is modified [7]. A fine balance between histone methyltransferase (HMT) and histone demethylase (HDM) activity makes histone methylation a highly dynamic and complex process [14, 15]. Moreover, DNA methylation and histone modifications have been shown to influence each other. A well-described crosstalk between DNA methylation and histone H3K9 methylation, mediated by the heterochromatin pro-

Figure 2: Environmental factors and epigenetic modifications. Several environmental stimuli are able to alter DNA methylation at gene promoters, posttranslational histone modifications and expression of noncoding RNAs. Adapted from Costantino et al. Eur Heart J. 2018;39(47):4150–8 [6].



tein 1 (HP1), represents a valid example of how histone modifications may facilitate the recruitment of enzymes (DNMT3a/b) involved in DNA methylation [16]. Another example is methyl-CpG binding protein 2 (MECP2), which recruits the histone methyltransferase SUV39H1 only after binding methylated DNA [17, 18].

Noncoding RNAs

ncRNAs are not transcribed into proteins but regulate gene expression by interacting with mRNA and proteins. Several studies in recent years have clearly shown the ability of ncRNAs to regulate gene expression in health and disease. Surprisingly, only a small portion (approximately 2%) of our genome is transcribed into proteins. Based on their length, ncRNAs can be subdivided into two groups: (i) small ncRNAs (sncRNAs, <200 nucleotides long) including microRNAs (miRNAs), and (ii) long ncRNAs (lncRNAs), which have a length of between 0.2 and 2 kb [19].

Epigenetic regulation of vascular function in obesity

A main feature of the obese vasculature is represented by the progressive loss of insulin sensitivity in the vascular endothelium [20]. Vascular insulin resistance is emerging as a powerful determinant of systemic insulin resistance and cardiometabolic disturbances. Endothelium-specific insulin resistance in *ApoE^{-/-}* mice leads to impaired NO signalling and aggravates atherosclerosis [21]. Of interest, endothelium-specific genetic disruption of the pro-inflammatory transcription factor NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B-cells) protects mice against insulin resistance in other insulin-sensitive organs, namely adipose tissue and skeletal muscle [22].

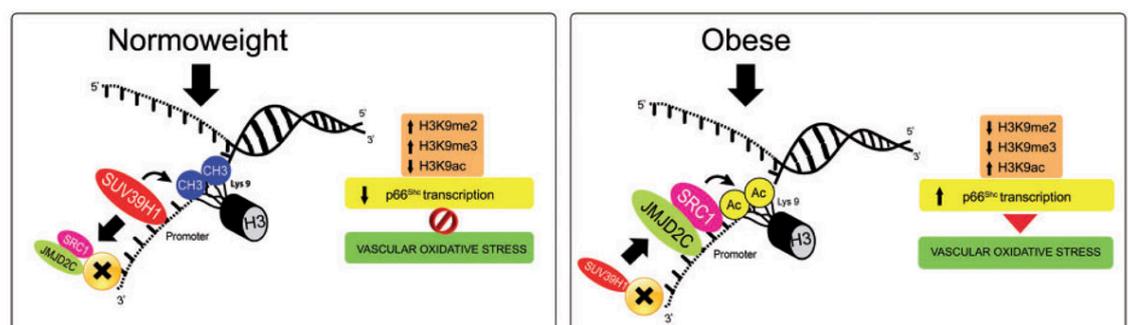
The key role of insulin resistance in the endothelium holds true in humans. Indeed, insulin response and subsequent endothelial nitric oxide synthase (eNOS) activation are blunted in freshly isolated endothelial cells from diabetic patients, whereas pharmacological blockade of protein kinase beta (PKC β) by LY379196 improved insulin signalling and restored eNOS activity [23]. Hence, endothelial insulin resistance may initiate metabolic alterations eventually leading to systemic impairment of insulin sensitivity [24]. Data from our lab have contributed to the finding that epigenetic signals underline alterations of in-

sulin signalling as well as vascular inflammation and oxidative stress. Specifically, a pro-oxidant protein – known as the mitochondrial adaptor $p66^{\text{Shc}}$ – critically participates in obesity-related changes of the vascular phenotype. In visceral fat arteries isolated from obese patients, $p66^{\text{Shc}}$ was upregulated and its expression correlated with oxidative stress, endothelial dysfunction and insulin resistance, as assessed with the homeostasis model assessment index (HOMA-IR) [25]. Unbiased epigenetic analyses showed that a complex network of chromatin enzymes, specifically the methyltransferase SUV39H1, the demethylase JMJD2C and the acetyltransferase steroid receptor coactivator-1 (SRC-1), were involved in the regulation of $p66^{\text{Shc}}$ gene expression by inducing an open chromatin via H3K9 demethylation and acetylation (fig. 3). Interestingly enough, *in vivo* and *in vitro* targeting of SUV39H1, JMJD2C and SRC-1 rescued obesity-induced endothelial dysfunction in mice by restoring NO bioavailability [25]. It has also been found that *in vivo* gene silencing of $p66^{\text{Shc}}$ restored endothelial insulin response by affecting the IRS-1/Akt/eNOS and NF- κ B pathways [26]. This set of experiments supports the notion that epigenetic editing of $p66^{\text{Shc}}$ promoter contributes to the development of endothelial insulin resistance and cardiometabolic traits in patients with obesity.

Epigenetic modifications and cardiovascular damage in type 2 diabetes

There is a clear link between high glucose levels and modifications of the epigenome. Hyperglycaemia is able to induce long-lasting epigenetic signals ultimately leading to persistent vascular damage [15]. Epigenetics may contribute to the explanation for the lack of benefit of intensive glycaemic control on cardiovascular complications in patients with T2D [27–29]. The perpetuation of vascular damage despite intensive glycaemic control is termed “metabolic” or “hyperglycaemic memory” [30]. Epigenetic modifications induced by hyperglycaemia are relatively stable over time and account for persistent alterations of transcriptional programmes implicated in vascular homeostasis. Fibroblasts isolated from T2D patients with foot ulcers display reduced methylation at the promoter of genes regulating blood vessel growth, wound repair and extracellular matrix remodelling [31]. Of interest, promot-

Figure 3: Role of chromatin modifying enzymes in obesity-induced vascular disease. A complex epigenetic machinery composed of SUV39H1, SRC-1 and JMJD2C is causally implicated in the transcriptional regulation of the pro-oxidant gene $p66^{\text{Shc}}$. Epigenetic-induced upregulation of $p66^{\text{Shc}}$ precipitates oxidative stress and endothelial dysfunction. H3K9me2 = histone 3 lysine 9 dimethylation; H3K9me3 = histone 3 lysine 9 trimethylation; H3K9ac = histone-3 acetylation. Adapted from Costantino et al. Eur Heart J. 2018;39(47):4150–8 [6].



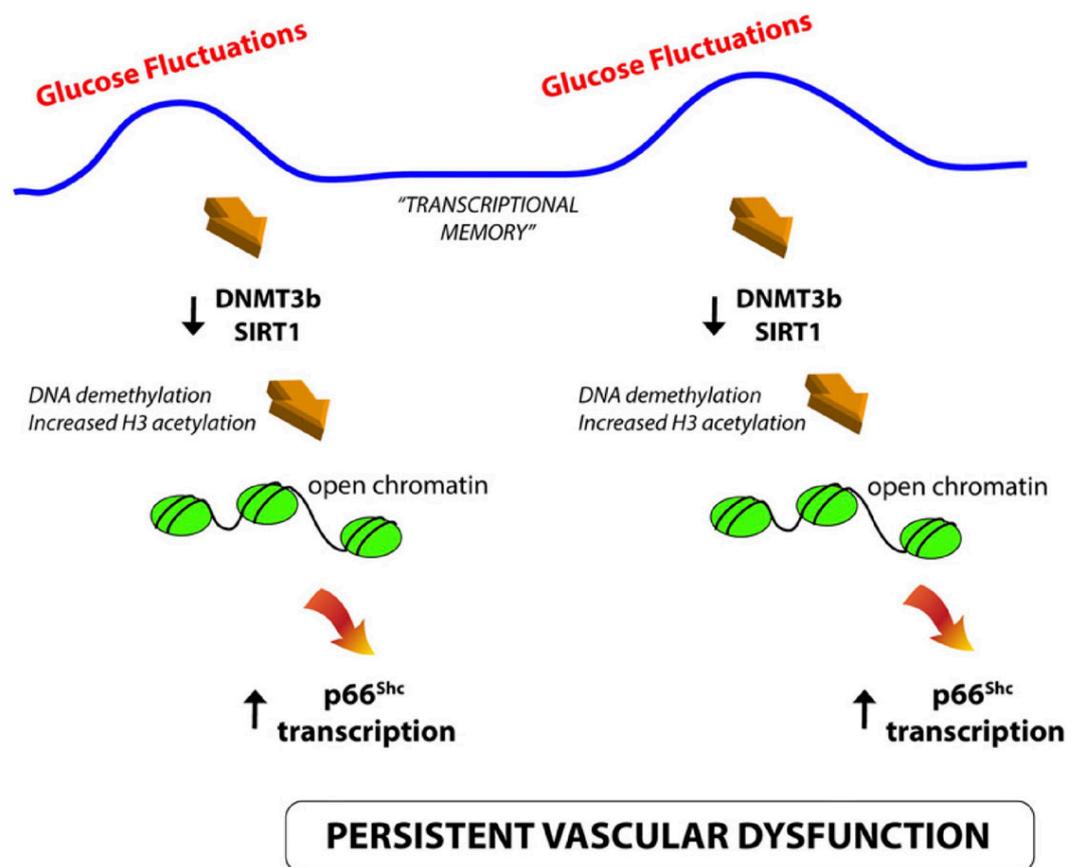
er demethylation in fibroblasts remained unchanged despite restoration of normoglycaemia. Hyperglycaemia was found to reduce DNA methylation at the promoter of the adaptor $p66^{Shc}$, thus leading to gene upregulation, cytochrome *c* oxidation and accumulation of free radicals [32]. Promoter demethylation was associated with acetylation of H3 on $p66^{Shc}$ promoter [32]. Persistent epigenetic changes of $p66^{Shc}$ were also reported in patients with diabetes. In this study, intensive glycaemic control for 6 months did not change DNA methylation and H3 acetylation of $p66^{Shc}$ promoter, thus leading to persistent endothelial dysfunction and oxidative stress, as assessed by brachial artery flow-mediated dilation and urinary levels of 8-isoprostane (8-isoPGF2 α). Moreover, it has been found that glucose excursions in diabetic patients were responsible for the persistent activation of DNA (cytosine-5-)-methyltransferase-3 β (DNMT3b) and sirtuin-1 (SIRT1), thus fostering an open chromatin and transcription of the pro-oxidant enzyme $p66^{Shc}$ (fig. 4) [33]. A further study showed that overexpression of SIRT1 was able to erase the hyperglycaemic memory by suppressing generation of reactive oxygen species (ROS). These effects were mediated by inhibition of NF- κ B/PARP and LKB1/AMPK pathways

[34] (PARP = poly(ADP-ribose)-polymerase 1; LKB1 = liver kinase B1; AMPK = AMP-activated protein kinase).

Reduced trimethylation of H3K9 was reported to foster the expression of pro-inflammatory genes in vascular smooth muscle cells (VSMCs) isolated from a mouse model of T2D [35]. Mono-methylation at lysine 4 of histone 3 (H3K4m) – a specific chromatin signature induced by the methyltransferase SETD7 – was found to promote inflammatory and pro-oxidant transcriptional programmes in the diabetic endothelium [36, 37]. The detrimental action of SETD7 was mainly explained by its ability to regulate the transcription of the NF- κ B subunit p65, which in turn leads to upregulation of pro-atherosclerotic genes *VCAM-1* and *ICAM-1* [36]. A recent study from our group has shown that SETD7 expression is increased in peripheral blood monocytes from T2D patients, and significantly correlates with endothelial dysfunction, inflammation and oxidative stress levels [37]. Importantly, several compounds have already shown the ability to inhibit SETD7 and may represent a potential therapeutic strategy to attenuate or prevent vascular inflammation and atherosclerosis [9].

Emerging evidence also support the involvement of ncRNAs in vascular and cardiac hyperglycaemic memory. Pro-

Figure 4: Role of glycaemic variability in persistent vascular dysfunction. In patients with T2D with target HbA1c values, continuous glucose fluctuations cause downregulation of chromatin-modifying enzymes DNMT3b and SIRT1 and subsequent epigenetic changes, namely reduced DNA methylation and increased H3 acetylation. Such epigenetic marks favour an open chromatin, leading to enhanced $p66^{Shc}$ transcription, oxidative burst, and persistent vascular dysfunction despite IGC. Therefore, glycaemic variability maintains an epigenetic-driven transcriptional memory that may contribute to the progression of diabetic vascular complications in this setting. Adapted from Costantino et al. Diabetes. 2017;66:2472–82 [33].



filing of ncRNAs in the diabetic mouse heart showed that 316 miRNAs were differentially expressed as compared with non-diabetic hearts [38]. Of note, the expression of 268 miRNAs remained persistently altered despite 3 weeks of intensive glycaemic control with insulin implants. The majority of dysregulated miRNAs were found to be involved in the regulation of myocardial hypertrophy, fibrosis, redox signalling and autophagy [38]. Moreover, it has been recently shown that miR-34 and miR-218, targeting SIRT1 and DNMT3b, respectively, are actively involved in chromatin remodelling, thus leading to persistent transcription of pro-oxidant genes in the heart of diabetic mice despite intensive glycaemic control [39]. These findings support the notion that ncRNAs may indirectly regulate chromatin activity, thus leading to enhanced gene expression. Altered expression of miR-125b, miR-146a-5p and miR-29a-3p in the vascular endothelium leads to persistent inflammatory signatures mainly via epigenetic modulation of NF- κ B, tumour necrosis factor (TNF)- α -induced protein 3 (TNFAIP3), TNF receptor-associated factor 6 (TRAF6) and interleukin-1 receptor (IL-1R) [40]. Persistent upregulation of miR-23b-3p in retinal endothelial cells was found to play a detrimental role in the pathogenesis of diabetic retinopathy by regulating SIRT1/NF- κ B pathway [41]. MiR-326 – a direct regulator of adiponectin and adiponectin receptors (ADIPOR-1, ADIPOR-2) – was persistently elevated in patients with T2D, despite 12 months of intensive glycaemic control. Suppression of adiponectin signalling by miR-326 may represent an important mechanism implicated in the pathogenesis of cardiometabolic disturbances, even in patients with optimal glycaemic control [42].

Toward epigenetic-based therapies

Taken together, the evidence discussed in this review indicates that plastic modifications of chromatin are causally implicated in the regulation of genes involved in the pathogenesis of cardiovascular complications in the setting of obesity and T2D. Although previous work has contributed to the deciphering of detrimental epigenetic networks in the heart and the vasculature, we are still far from a thorough comprehension of epigenetic modifications and their link with tissue-specific transcriptional programmes. The latter aspect is particularly important for the design of therapies aimed at erasing epigenetic signals only in specific cell types, namely endothelial cells, VSMCs and cardiomyocytes. Targeted epigenetic therapies would also avoid undesirable effects related to modulation of gene expression in other tissues. We currently have at our disposal several compounds able to modulate epigenetic modifications [9]. Of note, several chromatin modifying drugs, such as vorinostat and valproic acid, have been approved by the US Food and Drug Administration for the treatment of several conditions including cancer, neurological and CVD. Vorinostat and thichostatin A are histone deacetylase inhibitors (HDACs) with proven efficacy in preventing endothelial dysfunction, diabetic nephropathy and ischaemia/reperfusion myocardial injury in experimental models [43, 44]. Activation of SIRT1 by resveratrol also exert beneficial effects by rescuing obesity- and T2D-related endothelial dysfunction and insulin resistance [45, 46]. Future studies are warranted to investigate whether targeting

epigenetic networks may represent a potential strategy to reduce the burden of cardiovascular disease in the cardiometabolic patient.

Disclosure statement

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