Clinical relevance of epigenetic dysregulation in chronic kidney disease-associated cardiovascular disease

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ABSTRACT

Across the spectrum of clinical medicine, the field of epigenetics has gained substantial scientific interest in recent years. Epigenetics refers to modifications in gene expression which are not explained by changes in DNA sequence. Classical components of epigenetic regulation comprise DNA methylation, histone modifications and RNA interference. In chronic kidney disease (CKD), several features of uraemia, such as hyperhomocysteinemia and inflammation, may contribute to changes in epigenetic gene regulation. It has been suggested that these changes may affect genes related to cardiovascular disease. Thereby, a uraemia-associated disturbance in epigenetic regulation may contribute to the substantial increase in cardiovascular morbidity in CKD patients. The present review aims to summarize current knowledge of epigenetic dysregulation in cardiovascular disease from a nephrological perspective, with a special focus on DNA methylation. We first describe the impact of altered epigenetic regulation in non-CKD-associated arteriosclerosis, and next characterize uraemic features which may affect epigenetic gene regulation in the context of cardiovascular disease. Finally, we conclude that substantial additional work is needed before epigenetic regulatory mechanisms may become therapeutic targets in CKD-associated cardiovascular disease.

INTRODUCTION

Patients with chronic kidney disease (CKD) have an unacceptably high risk for cardiovascular events, which is mainly attributable to dramatically accelerated vascular disease. Traditional cardiovascular risk factors, such as hypercholesterolaemia, hyperglycaemia, arterial hypertension, smoking, obesity and physical inactivity, can only partly explain this high cardiovascular risk [1]. In line, conventional cardiovascular treatment strategies failed to improve cardiovascular survival in CKD patients substantially [1, 2].

Thus, future therapies will have to focus on non-classical cardiovascular risk factors, among which uraemia-associated alterations in epigenetic gene regulation attracted some interest in recent years. Epigenetic mechanisms are crucial regulators of cellular homeostasis, which control gene expression and maintain cell identity during subsequent cell divisions. Consequently, dysfunctional epigenetic gene regulation may substantially contribute to the onset and progression of diverse pathologies such as cancer or arteriosclerosis.

It is increasingly recognized that environmental factors may influence epigenetic regulation. In line, it is common opinion that in CKD patients, long-term exposure to the unphysiological uraemic milieu may affect epigenetic mechanisms, which may eventually comprise the regulation of arteriosclerosis-related genes.

The present review summarizes recent findings in the field of epigenetics regarding arteriosclerosis and CKD. Notably, epigenetic dysregulation may affect many other aspects of renal medicine not covered in this article. Starting in early life, when maternal–foetal epigenetic interactions are of paramount importance for human development [3], epigenetic mechanisms are of essential importance for renal physiology, and their dysregulation may induce and perpetuate renal disease [4].

Admittedly, research on epigenetic regulation in CKD is still a very evolving field in its very beginnings, whereas epigenetics has been extensively studied in other disciplines of internal medicine. In oncology, epigenetic research has entered the scene as early as 1983, when Feinberg and Vogelstein first demonstrated epigenetic dysregulation in cancer cells [5]. Since then, a steady increase in the understanding of epigenetic mechanisms in the pathogenesis of human cancer allowed to define new therapeutic strategies in oncology (summarized in [6–8]). In 2004, the Food and Drug Administration
approved the DNA methyltransferase inhibitor azacitidine for treatment of subtypes of myelodysplastic syndrome (MDS), where it proved to prolong survival compared with standard care [9]. Thus, adequate understanding of epigenetic dysregulation in human disease may allow to improve outcome in affected patients.

**DNA METHYLATION AS A CENTRAL EPIGENETIC REGULATOR**

The term ‘epigenetics’ refers to changes in gene expression which are caused by altered DNA accessibility without affecting the nucleotide sequence. In contrast to mutations in the DNA sequence, epigenetic marks are dynamic; they can be altered by exogenous factors including nutrition and environmental influences. Nonetheless, epigenetic mechanisms are sufficiently stable to transmit information on gene expression from one cell generation to the next; they control diverse biological phenomena such as X-chromosome inactivation, silencing of transposable elements or genomic imprinting [10].

Epigenetic mechanisms comprise several levels of gene regulation, which include DNA methylation, histone modifications and RNA interference. The present review will focus on DNA methylation, which is the best understood epigenetic modification in the context of CKD. For space constraints, the role of histone modifications and RNA interference in epigenetic regulation are not explained in detail, as excellent reviews have been published recently, to which the interested reader may refer to [11–13].

In mammals, DNA methylation occurs at the C5 position of cytosines predominantly in the context of CpG dinucleotides (cytosines followed by guanines). The 5′ regulatory regions of many genes are enriched in CpG dinucleotides which form so-called CpG islands, and their methylation generally prevents gene transcription. In contrast, transcriptionally active DNA regions are typically unmethylated (Figure 1) [14]. This mechanism of activation and repression of gene transcription by differential DNA methylation is mediated via alterations in chromatin configuration and accessibility of the transcription machinery to the promoter region.

DNA is associated with histones and other chromosomal proteins, which themselves can also be modified. Different modifications of histones exist such as acetylation or methylation, which regulate the degree of chromatin condensation and consequently the level of transcription. Of note, a cross-talk between DNA methylation and histone modifications exists, so that silencing of gene expression by DNA methylation is often associated with, e.g., deacetylation of histones in the same genomic region (Figure 1).

**DNA METHYLATION AND ARTERIOSCLEROSIS IN NON-CKD**

Within the spectrum of arteriosclerotic vascular disease, atherosclerosis defines a chronic inflammatory process characterized by endothelial cell activation, by infiltration of circulating monocytes and other leukocytes into the subendothelial space, by subsequent differentiation of monocytes towards macrophages and dendritic cells and by migration and proliferation of smooth muscle cells (SMCs) (Figure 2). Thus, transformation of several distinct cell types essentially contributes to the development of atherosclerosis (atherogenesis), and these cellular transformations necessarily require reprogramming of gene expression. Therefore, it is highly probable that epigenetic mechanisms may be centrally involved in atherosclerosis.

In recent years, several clinical and experimental studies analysed the implications of disturbed DNA methylation in atherosclerosis. Until now, only few studies focussed on CKD patients. All the more, a critical appraisal even of non-CKD studies appears fruitful to the nephrological community, augmenting our understanding of potential implications of epigenetic regulation in accelerated arteriosclerosis. In the following, we first discuss human studies which analysed global DNA methylation rather than methylation of specific genes. Secondly, we review human studies on gene-specific DNA methylation. Finally, we summarize human studies that assessed global or gene-specific DNA methylation in the context of CKD.

**GLOBAL ASSESSMENT OF DNA METHYLATION IN ARTERIOSCLEROSIS**

Global DNA methylation in cardiovascular disease has been assessed by analysing either peripheral blood cells, or vascular tissue. A small cohort study reported lower DNA methylation in peripheral blood cells (measured by cytosine extension assay) among 17 male patients with prevalent cardiovascular disease compared with 15 male healthy controls [15]. Such an association between global DNA hypomethylation and prevalent cardiovascular disease was confirmed in the larger Normative Aging Study, when analysing DNA methylation of long interspersed nucleotide element-1 (LINE-1) repetitive elements in peripheral blood mononuclear cells as a marker for global DNA methylation among 712 elderly men [16]. Moreover, lower LINE-1 methylation at study initiation predicted cardiovascular mortality in this cohort.

Contrarily, global DNA hypermethylation rather than hypomethylation in peripheral blood cells was reported among 137 Indian coronary artery disease (CAD) patients compared with 150 controls (assessing global DNA methylation by cytosine extension assay) [17], and among 101 Singapore Chinese Health Study participants with prevalent myocardial infarction and/or stroke compared with 185 controls (assessing leukocyte DNA methylation of repetitive elements as global DNA methylation marker) [18]. In the latter study, male patients with incident cardiovascular events during follow-up had higher global DNA methylation than subjects with event-free survival, again contrasting to findings from the Normative Aging Study.

When globally analysing DNA methylation in vascular tissue samples rather than in peripheral leukocytes, current
FIGURE 1: Mechanism of epigenetic gene regulation. Two major components of epigenetic regulation are DNA methylation and histone modifications. (1) DNA methylation occurs at the C5 position of cytosines in the context of CpG dinucleotides (indicated as filled circles). An open chromatin structure (feature of transcriptionally active genes) is characterized by unmethylated CpGs (indicated by open circles). (2) Further regulatory mechanisms are provided by histone modifications, which may either allow ('permissive modifications') or silence ('repressive modifications') gene transcription. Of note, interactions between both regulatory pathways exist.

FIGURE 2: DNA methylation in atherosclerosis. Following initial endothelial activation, circulating monocytes and other leukocyte subsets are recruited into the subendothelial space. There, monocytes differentiate into macrophages, which take up lipids to form foam cells and thus give rise to fatty streaks, which are the earliest ultrastructural alterations in atherosclerosis. These early atherogenic lesions may subsequently gradually develop into advanced atherosclerotic plaques, which are characterized by a lipid- and macrophage-rich necrotic core; migration of SMCs from the tunica media into the tunica intima may further contribute to this atherogenic process. Current epigenetic knowledge from analyses of global and/or gene-specific DNA methylation suggests alterations (comprising both hypo- and hypermethylation) to occur in early as well as in advanced atherosclerotic lesions. Moreover, epigenetic changes in peripheral blood leukocytes were characterized in the context of atherosclerosis, which may become a clinical marker for epigenetic dysregulation in subjects at cardiovascular risk.
data point towards an association between atherosclerotic lesions and hypo- rather than hypermethylation. With a microarray-based approach, Castillo-Díaz et al. [19] compared 45 human atherosclerotic coronary artery samples from patients undergoing revascularization surgery and 16 control aortic fragments from patients undergoing aortic valve replacement; a near-complete demethylation of normally hypermethylated CpG islands was found in advanced human atherosclerotic lesions. In line, Hiltunen et al. [20], who analysed DNA methylation via high performance liquid chromatography in 55 human arterial samples obtained from autopsy or amputation, found reduced global DNA methylation in advanced atherosclerotic arteries compared with normal arteries.

These human studies on epigenetic dysregulation in cardiovascular disease are complemented with data from animal models. DNA hypomethylation was detected in atherosclerotic lesions from New Zealand White rabbits and from ApoE knock-out mice on a Western-type diet [20, 21]. Interestingly, similar DNA hypomethylation occurred during rabbit aortic SMC transdifferentiation [20]. Further animal data point to very early dysregulation of DNA methylation in atherogenesis, as differential DNA methylation—both hypomethylation and hypermethylation—is present in aortas and PBMCs of ApoE−/− mice before any signs of atherosclerotic lesions [22]; thus, changes in DNA methylation may serve as very early markers of atherosclerotic vascular disease.

Presently, it remains enigmatic why some reports suggested DNA hypomethylation in human atherosclerosis, while other studies yielded contradictory findings. Differences in study size, definition of atherosclerotic, respectively, atherosclerotic disease and different technical approaches for assessment of global DNA methylation may partly contribute to these discrepancies. It is beyond the scope of the present review to critically discuss limitations of different methods for methylation analysis in detail. Taken together, only application of sophisticated methods for analysis of DNA methylation, which may be standardized across different laboratories, and recruitment of subjects from well-characterized cohorts will provide robust data that may confirm the importance of epigenetic dysregulation in the pathogenesis of cardiovascular disease.

However, the most notable limitation of current studies is the fact that any measurement of global DNA methylation will inevitably provide an oversimplified assessment of epigenetic dysregulation, as it neither quantitatively nor qualitatively acknowledges the co-existence of hypo- and hypermethylation of distinct genes within the same cell. In line, it has been suggested earlier that hypermethylation of atherosclerosis-protective and hypomethylation of atherosclerosis-susceptible genes may exist in atherosclerotic disease [12, 23–25].

Thus, although changes in global DNA methylation status may point towards a pathological condition, a better understanding of the interplay between epigenetic dysregulation and accelerated atherosclerosis mandates DNA methylation analyses of specific genes.

Most studies on site-specific epigenetic regulation in atherosclerosis somewhat arbitrarily selected single genes and reported their methylation status.

In this context, methylation of oestrogen receptor α and β genes (ERα and ERβ) was investigated repeatedly. Oestrogen receptors are present in SMCs and endothelial cells, where they may mediate vasculoprotective effects of oestrogens. Hypermethylation of the promoter regions of the ERα and ERβ genes was detected in coronary plaques and—more specifically—in SMCs during their transformation from a normal to a proliferative state [26–28]. Similarly, hypermethylation of monocarboxylate transporter 3 (MCT3) was found in transforming SMCs [29].

Moreover, in human atherosclerotic lesions, hypomethylation of the promoter region of the 15-lipoxygenase gene [20], and hypermethylation of the tissue factor pathway inhibitor-2 (TFPI-2) [30] were described.

Beyond such single-gene analyses, Castillo-Díaz et al. [19] performed a broader, microarray-based approach that revealed a total number of 142 hypomethylated and 17 hypermethylated CpG islands in human atherosclerotic arteries. Many of these CpG islands could be linked to genes coding for signalling and transcription factors such as PROX1, NOTCH1 or FOXP1, while others were annotated to genes connected to angiogenesis, SMC modulation and inflammation.

Further research is clearly needed to confirm and expand results of these pioneering studies, before specific epigenetic biomarkers for atherosclerosis may be defined on a more solid basis. Next, it will have to be tested in how far experimental data from tissue analysis are mirrored by similar changes in samples which can be obtained in convenient, less invasive manner—such as circulating leukocytes—before these experimental findings may become clinically relevant.

General consensus exists by most experts that the toxic uraemic milieu may exert a crucial impact on epigenetic gene regulation and may thus perpetuate CKD-associated accelerated atherosclerosis [12, 23–25, 31, 32]. Nevertheless, surprisingly few experimental and clinical studies on this topic have been reported, most of which analysed altered DNA methylation in the context of CKD-associated hyperhomocysteinemia [33] and inflammation [34].

Homocysteine is a central component of the one-carbon metabolism, which regulates DNA methylation. Its
derivative S-adenosylmethionine (SAM) is the universal methyl group donor for >100 different cellular methylation reactions, including DNA methylation (Figure 3). After transfer of a methyl group to its target, SAM is converted to S-adenosylhomocysteine (SAH), which is a powerful competitive inhibitor of SAM-dependent methyltransferases. Therefore, efficient removal of SAH is essential for cellular methylation reactions, which require hydrolysis of SAH into homocysteine and adenosine via SAH hydrolyases. Importantly, this reaction is reversible, with the equilibrium favouring SAH formation rather than its hydrolysis, and only rapid removal of homocysteine and adenosine allows this reaction to proceed in the hydrolytic direction. In contrast, any accumulation of homocysteine would directly increase SAH levels and thereby subsequently inhibit transmethylation reactions. Homocysteine may either be removed through the remethylation pathway, in which methionine synthase (in a folate/vitamin B12-dependent reaction) or betaine-homocysteine methyltransferase (using betaine as methyl group donor) will convert homocysteine into methionine. Alternatively, homocysteine can undergo transsulfuration to cystathionine in a vitamin B6-dependent pathway (cystathionine-β-synthase).

In CKD, homocysteine levels are elevated because of both decreased renal excretion and impaired capacity to metabolize homocysteine. In clinical cohort studies, CKD patients with highest homocysteine levels suffered most cardiovascular events [35]. This inspired numerous groups to explore possible pathophysiological pathways which underlie such detrimental effects of hyperhomocysteinemia. Earlier studies first focussed on functional pathways, such as production of reactive oxygen species [36], promotion of leukocyte recruitment [37] and SMC proliferation [38], as well as induction of a pro-thrombotic state [39]. Only in the last years, the implications of homocysteine in epigenetic mechanisms came into scientific focus.

Ingrosso et al. [33] were the first to analyse DNA methylation in the context of CKD-associated hyperhomocysteinemia. Assessing global DNA methylation by cytosine extension assay and southern blotting, 32 male hyperhomocysteinemic haemodialysis patients were found to have significantly lower DNA methylation compared with 11 healthy controls. Moreover, in haemodialysis patients, DNA hypomethylation correlated with plasma homocysteine concentrations, and folate therapy partly restored DNA methylation.

In contrast, Nanayakkara et al. [40] failed to reproduce the association between homocysteine and DNA methylation in 93 patients with less advanced CKD (CKD stage 2–4), when analysing global leukocyte DNA methylation by tandem mass spectrometry. Nor did they find an association of global DNA methylation with renal function, subclinical arteriosclerosis (measured as common carotid intima media thickness) and endothelial function. Additionally, homocysteine-lowering vitamin treatment had no effect on global DNA methylation. These discrepant findings may partly be explained by different

**Figure 3:** Role of homocysteine metabolism in methylation reactions. The homocysteine derivative SAM is the universal methyl group donor for a multitude of different methylation reactions, such as DNA methylation. After transfer of its methyl group, S-adenosylhomocysteine (SAH) is formed, which is a competitive inhibitor of methyltransferases. Removal of SAH requires its hydrolysis into homocysteine and adenosine. Since this reaction is reversible, rapid removal of homocysteine is crucial, as accumulation of SAH may inhibit cellular methylation reactions. This removal is achieved either via the remethylation or via the transsulfuration pathway.
study designs, as a folate wash-out period was mandatory in the study by Ingrosso et al. [33], but not in the latter trial [40].

Finally, our group measured parameters of the one-carbon metabolism and leukocyte LINE-1 methylation as a surrogate marker of global DNA methylation in 22 haemodialysis patients and 26 healthy, age- and sex-matched controls. Surprisingly, haemodialysis patients had higher rather than lower LINE-1 methylation compared with controls [41]; a correlation between SAH and LINE-1 methylation was neither found within the cohort of haemodialysis patients, nor within the group of healthy controls. Admittedly, this study is limited by the small study cohort size, and by the use of a rather crude surrogate marker for estimation of global DNA methylation.

In summary, the use of differing laboratory techniques, and diverse study designs, yielded controversial data on the impact of a disturbed one-carbon metabolism on DNA methylation in CKD. In our opinion, a broader understanding will require more refined methodological approaches, which should comprise analysis of gene specific rather than global DNA methylation. In this regard, a focus on arteriosclerosis-related genes appears worthwhile.

From a clinical point of view, it may be argued that further analyses of the implications of one-carbon metabolism on DNA methylation in CKD seems futile, since several interventional trials which aimed to attenuate hyperhomocysteinemia in CKD via supplementation of folate, vitamin B₆ and/or B₁₂ failed to affect cardiovascular morbidity in CKD patients [42–46].

We nevertheless reckon that these disappointing results do not preclude a pathophysiological role of one-carbon metabolism in cardiovascular disease: while supplementation with folate, vitamin B₆ and/or B₁₂ may reduce homocysteine levels in CKD, it fails to affect plasma SAH [47]. This is of particular interest for two reasons: first SAH rather than homocysteine is increasingly considered the real culprit in cardiovascular disease [48, 49], as SAH, but not homocysteine, directly inhibits methylation reactions. Secondly, SAH accumulates in excess to homocysteine with declining renal function, given that the kidney is the major site of SAH disposal in humans [50, 51].

Inflammation, Cardiovascular Disease and DNA Methylation in CKD

A second focus of epigenetic research in CKD centres on inflammation-induced disturbances in DNA methylation. Chronic (micro)inflammation is a common feature in CKD, which drives the development and progression of atherosclerotic lesions and thus contributes to elevated cardiovascular morbidity and mortality in CKD patients [52].

Beyond the field of nephrology, several studies suggested chronic inflammation to trigger DNA hypermethylation a decade ago [53, 54]; in line, proinflammatory cytokines were shown to regulate a DNA methyltransferase gene [55].

In CKD, Stenvinkel et al. [34] assessed global DNA methylation in peripheral blood leukocytes from 37 patients in CKD stages 3 and 4, 98 incident dialysis patients, 20 prevalent haemodialysis patients and 36 controls by the Luminometric Methylation Assay (LUMA) method. When patients were subdivided into inflamed (CRP ≥ 10 mg/L) and noninflamed (CRP < 10 mg/L) groups, inflamed patients (n = 62) had significantly higher global DNA methylation than noninflamed patients (n = 93) or controls. When incident dialysis patients were followed for 36 ± 2 months, DNA hypermethylation was significantly associated with all-cause and cardiovascular mortality.

A first study on gene-specific DNA methylation analysis in the context of inflammation and CKD focussed on DNA methylation of the p66Shc (SHC1) gene [41]. p66Shc is a stress response protein involved in reactive oxygen species metabolism. In murine studies, p66Shc deletion renders resistance to oxidative stress, thus prolonging life span and protecting against age-related endothelial dysfunction [56, 57]. Recruiting 22 haemodialysis patients and 26 controls, we found that the p66Shc gene is hypomethylated in human CKD, which may lead to enhanced expression of this gene and subsequently contribute to oxidative stress-mediated arteriosclerosis in CKD.

Admittedly, more comprehensive data on epigenetic dysregulation in the context of CKD-associated inflammation are needed, which should provide both more specific information than global methylation analysis, and a broader data set than analysis of (arbitrarily selected) single-gene methylation.

DNA Methylation Profiling in CKD

Against this background, two recent studies aimed to identify epigenetic biomarkers in CKD in a whole genome approach. Using the Illumina HumanMethylation27 Bead Chip array, Sapienza et al. [58] performed DNA methylation profiling in 24 diabetic haemodialysis patients and 24 diabetic patients without diabetic nephropathy. After extracting DNA from saliva, methylation was measured at 27578 CpG sites, which allowed to analyse methylation of >14 000 genes. One hundred and eighty-seven of these genes were found to be differentially methylated at least at two CpG sites, many of which were implicated in diabetic nephropathy and/or kidney disease; in pathway analysis, these differentially methylated genes could be linked to inflammation, oxidative stress, ubiquitination, fibrosis and drug metabolism. Of note, this study deliberately aimed to focus on the identification of epigenetic biomarkers for kidney disease rather than on the characterization of dysregulated genes in the context of CKD-associated arteriosclerosis.

Therefore, we set out to resolve this question by performing genome-wide DNA methylation analysis using SuperTAG methylation-specific digital karyotyping (SMSDK) in 10 male haemodialysis patients and 10 matched controls without kidney disease [59]. With this method we analysed 575744 loci, 4288 of which displayed...
Differential methylation with a P-value of $10^{-10}$. Differentially methylated genes were linked to distinct proatherogenic processes such as inflammation (e.g., TNFSF10, LY96, IFNGR1, HSPA1A, and IL12RB1), lipid metabolism and transport (e.g., HMGCR, SREBF1, LRPP, EPXH2, and FDX5), proliferation and cell-cycle regulation (e.g., MIK67, TP53, and ALOX12) as well as angiogenesis (e.g., ANGPT2, ADAMTS10, and FLT4). Importantly, by using the 'Genetic Association Database' we identified 52 genes which are associated with cardiovascular disease (e.g., HMGCR, TP53, ANGPT2, IFNGR1, and HSPA1A) and 97 genes with immune/infection diseases (e.g., TNFSF10, IL12RB1, MMP24, CASP8, and SPN). These results point for the first time towards a dysregulation of arteriosclerosis-related genes in CKD, and may thus indicate an implication of epigenetic dysregulation in accelerated arteriosclerosis in CKD.

CONCLUSIONS AND FUTURE PERSPECTIVES

After a significant contribution of epigenetic dysregulation to cardiovascular disease has been suggested in preliminary studies, the issue of potential therapeutic interventions arises.

Interestingly, unspecific epigenetic modifications have already entered contemporary cardiovascular medicine, as some of the pleiotropic effects of routinely used drugs have been attributed to epigenetic modifications [60]. However, a more tailored approach in cardiovascular medicine is still not in sight. Of note, in other fields of internal medicine, direct targeting of epigenetic regulatory mechanisms—e.g., by DNA methyltransferase inhibitors and histone deacetylase inhibitors—are already integrated in clinical medicine albeit at the price of unspecific intervention [60].

Against the background of the dramatically high cardiovascular disease burden, which cannot be satisfactorily lowered by conventional treatment strategies, transfer of these novel therapeutic avenues to the field of nephrology should become a research priority in the future. Given the present paucity of data in this field, we suggest the following next steps.

First, after gene-specific changes in DNA methylation have been characterized very recently, epidemiological studies should aim to characterize those site-specific epigenetic modifications that will predict cardiovascular events in a large cohort of CKD patients. Such approach would allow clinicians to identify high-risk individuals who may specifically benefit from preventive and therapeutic interventions. Even though such interventions are presently limited to conventional therapeutic strategies, comprising stringent blood pressure control and proteinuria lowering, more specific interventions into epigenetic regulatory pathways might become available in future years. Therefore, we aim to analyse prognostic implications of specific changes in DNA methylation of pre-defined genes among 444 CKD patients in our ongoing CARE FOR HOMe study.

Secondly, to characterize potential therapeutic approaches in epigenetic medicine, a better understanding of a disturbed C1-metabolism in CKD-associated cardiovascular disease is needed. After folate, vitamin B6 and/or B12 failed to improve the high cardiovascular risk in CKD patients despite lowering homocysteine levels, the role of other C1-metabolites such as SAH will gain substantial interest in forthcoming years. Against this background, we are studying the association between kidney function, C1-metabolites and cardiovascular disease in our epiGEN HOME project, comprising our I LIKE HOMe and CARE FOR HOMe trials. We postulate that elevated SAH may surpass homocysteine as cardiovascular outcome marker in CKD. If this hypothesis holds true, strategies to efficiently lower SAH levels in CKD patients should be explored.

Finally, future experimental and clinical studies should aim to explore further areas of epigenetic regulatory mechanisms beyond DNA methylation, including histone modifications and RNA interference, as both mechanisms still remain very poorly characterized in the context of CKD-associated cardiovascular disease.

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CONFLICT OF INTEREST STATEMENT

None declared.

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