Methyl donor nutrients in chronic kidney disease: Impact on the epigenetic landscape

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List of abbreviations and definitions:

CKD: chronic kidney disease
CVD: cardiovascular disease
SAM: S-adenosyl-L-methionine
DNMTs: DNA methyltransferases
Met: methionine
SMHT: serine hydroxymethyl transferase
MTHFR: methylenetetrahydrofolate reductase
CHDH: choline dehydrogenase
BHMT: betaine-homocysteine S-methytransferase
SAH: S-adenosylhomocysteine
Rasal1: RAS protein activator like 1
THF: 5,10-methyl-tetrahydrofolate
NF-KB: nuclear factor kappa B
eGFR: estimated glomerular filtration rate
NADPH: Dihydronicotinamide-adenine dinucleotide phosphate
Igf2: insulin-like growth factor *2*
Pparα: peroxisomal proliferator-activated receptor alpha
TMA: trimethylamine
TMAO: trimethylamine-N-oxide
FMO3: flavin mono-oxygenase 3

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Abstract

Epigenetic alterations, such as those linked to DNA methylation, may potentially provide molecular explanations for complications associated with altered gene expression in illnesses, such as chronic kidney disease (CKD). While both DNA hypo- and hypermethylation have been observed in the uremic milieu, this remains only a single aspect of the epigenetic landscape and, thus, of any biochemical dysregulation associated with CKD. Nevertheless, the role of uremia-promoting alterations on the epigenetic landscape regulating gene expression is still a novel and scarcely studied field. Though few studies have actually reported alterations of DNA methylation via methyl donor nutrients intake, emerging evidence indicates that nutritional modification of the microbiome can affect one carbon metabolism and the capacity to methylate the genome in CKD. In this review, we discuss about the nutritional modifications that may affect one carbon metabolism and, the possible impact of methyl donor nutrients on the microbiome, CKD and its phenotype.

Keywords: methyl donor nutrients, DNA methylation, chronic kidney disease

1. Introduction

Chronic kidney disease (CKD) with a global prevalence of 10-15% represents a public health problem worldwide (1). CKD patients present with many complications, including persistent low-grade inflammation and oxidative stress, which are important contributors to adverse outcomes, such as cardiovascular disease (CVD) (2). Inter-individual variation in disease progression and response to therapy remains substantial and the underlying factors contributing to this variation remain largely unknown. One potential source of this variation resides in epigenetic differences and in particular, the epigenetic landscape of ageing (3, 4), as CKD manifests as a disease of accelerated ageing (5). The epigenetic landscape of ageing refers to the interplay, over the life course, between the environment and canonical features of the genomic methylome and chromatin structure, along with non-canonical features, such as the co-ordinated regulation of a broad range of cellular biochemistry by non-coding RNAs (3,4,5).

Epigenetic regulation of the process of ageing is influenced directly by various factors, including nutrition, inflammation, the gut microbiome, psychosocial and lifestyle factors (3). Understanding how the epigenetic landscape changes in CKD would offer novel approaches to better understanding the uremic phenotype. For example, tailor-made interventions to target the underlying biochemistry of canonical epigenetic features via the nutritional acquisition of methyl donors required for maintenance of the methylome.

1.1 What is epigenetics and how is it regulated?

Epigenetics refers to heritable changes that are not coded for in the underlying DNA sequence. They enable a means of changing phenotype without changing the genotype. Epigenetic regulation of gene expression allows for rapid physiological

adaptations to environmental change, critical for development and homeostasis. In their canonical form, epigenetic modifications involve DNA methylation or histone modification (via methylation, acetylation, phosphorylation, ubiquitylation, and sumolyation) (3, 6). Such epigenetic changes regulate and coordinate access for transcriptional machinery to adjust gene expression, thus enabling changes in phenotype without changes in genotype. Additionally, at a non-canonical level, reciprocal regulatory networks of non-coding RNAs integrate canonical features within the greater epigenetic landscape (4, 6).

The activation and repression of gene transcription by DNA methylation can be influenced by a range of factors including uremia and metabolic features, such as hyperhomocysteinemia, oxidative stress and inflammation (3-7). There is a paucity of information available on the methylome in CKD, which demands that knowledge of exactly how, when, where and which genes are activated or repressed as a consequence of methylation-induced changes. Additionally, how the ensuing physiological changes occur, must be viewed in the context of the overall epigenetic landscape of ageing. Unsurprisingly, both global hypo- (8) and hyper-methylation (9) have been reported in CKD. While accelerated ageing inherent in CKD should typically result in genomic hypomethylation and acceleration of a methylation based epigenetic clock, individual genes may show hypermethylation of their promoters or other regulatory elements, reflective of the human genome encodes diminution/loss of expression and thus decreased functional activity (2, 3). However, the biological context of any equivocal reports indicating an increase in global methylation remains unknown and requires evaluation (10). Epigenetic studies in CKD are, thus, important for better understanding the variable and complex uremic phenotype (11).

Studies on epigenetic modifiers that can modulate features of the epigenetic landscape are scarce. Maintenance of the methylome is critical to the integrity of the epigenome. This is regulated via one carbon metabolism and, thus, in turn by nutritional input of methyl donors, such as Met, folate, vitamin B-12, choline and betaine which are substrate providers for many epigenetic processes (12, 13). Little is known about how nutrients affect epigenetic processes in CKD. Hence this review seeks to evaluate the evidence of epigenetic changes in CKD involving DNA methylation and, discuss the possible impact of methyl donor nutrients and their influence on the microbiome, as putative modifiers of CKD and its phenotype.

1.2 The principles and the evidence for methylation dynamics in CKD

DNA methylation, a dynamic and flexible means of modulating the response of the genome to environmental stimuli, is an inherent component of natural biological processes, such as ageing, that may reflect or possibly explain dysregulation in disease processes (13, 14). Typically, this comprises the transfer of a methyl group (CH3) from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA by DNA methyltransferases (DNMTs) enzymes, to form 5-methylcytosine (15, 16).

DNA methylation in mammals is highly regulated and DNMT activity can be modulated by numerous interactions with a diverse set of cofactors, post-translational modifications, alternative splicing and gene loss and duplication (15, 16, 17). DNA methylation patterns are considered the key markers of epigenetic programming and play an important role in maintaining genome integrity, disruption of which may result in chromosome instability. In human disease, altered DNA methylation patterns are one of the earliest and most consistent molecular changes observed (3, 18).

DNA methylation generally occurs within the context of CpG dinucleotides, where methylation permits methylation-binding proteins to bind to the site, repressing transcription via recruitment of chromatin remodeling factors. Most CpG sites within the mammalian genome are methylated, including CpGs found in and between genes (intronic and intergenic regions respectively) (18, 19). In contrast, regions which are enriched for CpG sites, termed "CpG islands," are commonly depleted of methylated DNA, allowing an open chromatin structure and binding of transcription factors. CpG islands are highly conserved between mice and humans and approximately 70% of all gene promoters are found in CpG islands (4) (Figure 1).

Regulation of DNMT activity can be influenced directly by nutrition via one carbon metabolism. Nutritionally acquired Met, for example, is a direct precursor for SAM, a universal methyl donor for several transmethylation pathways involving dietary nutrient-dependent enzymes (Figure 2). These include serine hydroxymethyl transferase (SMHT), with vitamin B-6 as a cofactor, methylenetetra-hydrofolate reductase (MTHFR) with vitamin riboflavin as a cofactor, Met synthase with vitamin B-12 as a cofactor, choline dehydrogenase (CHDH) with vitamin choline as cofactor and betaine homocysteine methyltransferase (BHMT) with betaine as a cofactor, After a methyl group is removed from SAM by one of the respective DNMTs, S-adenosyl-homocysteine (SAH) is formed by the action of SAH hydrolase; this is hydrolyzed to homocysteine, which then enters into Met cycle (discussed below) (14, 18). In this way, SAH competes with the activity of DNMTs and acts as a powerful competitive inhibitor of SAM. Consequently, it plays an important role in maintenance of the cellular methylome (7, 19).

In CKD, dynamic methylation at CpG islands is an inherent feature of epigenetic regulation, observed *in vitro*, in pre-clinical animal models and human studies (4, 20,

21, 22, 23). Notable examples of such regulation pertinent to renal biology, include hypermethylation of the RAS protein activator like 1 (*Rasal1*) gene (encoding an inhibitor of the Ras oncoprotein) causing kidney fibrosis in mice and suppression of Klotho activity (considered a regulator of ageing) through hypermethylation induced by microbial derived uremic toxins, such as indoxyl sulfate and *p*-cresyl sulfate (20). Notably, renal fibrosis has also been linked to the presence of senescent renal cells in CKD and links changes in the epigenetic landscape of ageing to pathological features of the disease (21).

Epigenetic change in CKD is further exemplified by the *MTHFR* gene. Its enzymatic product is MTHFR, which promotes methyl radical synthesis in the homocysteine cycle, and can provide methyl groups for DNA methylation. MTHFR catalyzes the reduction of 5,10-methyl-tetrahydrofolate (THF) to 5-methyl-THF, in order to form Met from homocysteine, the concentration of which increases in CKD and is associated with increased CVD risk (22). CKD patients also display a significant up-regulation in the methylation at the MTHFR promoter, commensurate with decreased production of this enzyme (22). In turn, this is expected to contribute to a loss of global genomic methylation correlating with increasing biological age (13, 16).

In practice, however, extrapolating methylation changes at a given locus to a global picture of the epigenome in the uremic environment is challenging and complex. Differences in methodology and in techniques for assessment of DNA methylation changes have contributed to equivocal reports when applied to such analyses in CKD cohorts (7). Both Zinellu et al. (8) and Nanayakkara et al. (23) have demonstrated DNA hypomethylation in whole blood from CKD patients, while Hsu et al. (24), observed no such methylation loss, but did observe reduced DNMT3b transcription, supporting loss of regulation of the methylome with age and disease. In contrast, Stenvinkel et al. (9)

reported that inflamed CKD patients exhibited global DNA hypermethylation, which was associated with CVD and increased mortality. Recently, Ghigolea et al. (25), evaluating 80 haemodialysis (HD) patients, also reported global DNA hypermethylation in whole blood in dialysis patients compared to healthy individuals.

Evaluation of renal function associated with methylome changes in the general population has also identified a strong association with genes involved in ageing processes. Bomotti et al. (26) investigated the methylation status of 14,000 genes and their relationship with eGFR in the GENOA Study. The top ranked candidates showing significant methylation changes correlating with variation in kidney function were involved in regulating the ageing process and inflammation. Notably, a high rank was observed for Krüppel-like transcription factor 2, which has a role in regulating blood flow through the glomerular kidney bed and regulation of most of the nuclear factor kappa B (NF-KB)-mediated activities, including inflammatory and fibrotic processes (27). This provides a rational basis for linking changes in epigenetic status with renal function in CKD. However, a more recent epigenome wide association study of kidney function and CKD in 4859 participants from the general population only identified DNA methylation changes at 19 CpGs that were associated with estimated glomerular filtration rate (eGFR) or CKD at epigenome-wide significance (28). This indicates the complexity in evaluating the directional nature of any methylation dynamics in CKD, relating to cause, effect and ageing.

1.3 How might nutrition impact on the epigenome in CKD?

As changes to the methylome are, context dependent, typically in response to dynamic environmental cues, it is worth discussing the impact of nutrition and how this may lead to observed differences in distinct clinical cohorts (3, 4, 5). A range of

nutritional factors feeding into one carbon metabolism will be discussed and evaluated for their capacity to influence changes in the epigenetic landscape.

2. Methyl Donor Nutrients

Methyl donor nutrients, such as Met, folate, vitamin B-12, choline and betaine are substrate providers for many epigenetic processes (29). For example, both maternal folate and choline supplementation during pregnancy can cause epigenetic alterations to genes in offspring (30). While most studies show positive health-effects associated with methyl donor supplementation, mounting evidence has also indicated the potential for deleterious effects, including an increased risk of cancer and neurological disorders (31, 32). The impact of dietary methyl donors in CKD remains to be fully elucidated. It is, thus, pertinent to discuss the relative and respective merits of nutritionally derived methyl donors in the context of uremia.

2.1 Methionine

Met, an essential Sulphur-containing amino acid, is a central molecule in one-carbon metabolism. Maintaining an adequate level of Met derived via nutritional intake, 19 mg/kg/day of methionine + cysteine according to Institute of Medicine (IOM, 2005), is essential for ensuring an appropriate level to enable sufficient DNA methylation, facilitated by SAM production. Variation of the amount of Met in the diet can influence DNA methylation levels and consequently contribute to the dysregulation of gene expression (33).

Red meat is predominantly abundant in Met content per total protein content, and high frequency of red meat consumption is associated with accelerated ageing and diminished renal function in man (34) and in animals (35). Met restriction can be

achieved through a vegan diet, however as Met is an essential amino acid it cannot be entirely removed from diet (36). Restriction of Met may extend longevity (37), improve glucose and lipid metabolism and reduce oxidative stress (38). This postulate is supported by a range of *in vitro* and *in vivo* pre-clinical models, indicating altered methylation profiles in a variety of human diseases (39, 40), that can be directly affected by altered Met levels in the diet (40).

Met restriction is particularly pertinent to renal biology. Indeed, Cooke *et al*. (41) recently showed that kidneys in 5/6 nephrectomized mice play an important role in maintaining osmotic balance during Met restriction diet, by up-regulating genes involved with ion transport. Additionally, Met restriction may delay the progression of CKD by down-regulating inflammatory and fibrotic processes. This results in lower expression of urinary biomarkers normally elevated during kidney disease (41). Correspondingly therefore, a high Met diet may induce elevated levels of oxidative stress and elevate renal damage in kidneys with tubular hypertrophy (42,43). Surprisingly therefore, , Amaral *et al*. (44) have reported that a high Met diet is not deleterious to kidney cells in Wistar rats. Furthermore, dietary Met supplementation did not alter the SAM/SAH ratio, nor DNA methylation at the promoter region of the tumor suppressor gene p53. However, it did result in restoration of glutathione levels in animals treated with doxorubicin.

2.2 Folate

Folate is the term used to describe a range of forms of the water-soluble vitamin B-9 that occurs naturally in foods (Table 1) (41,45). Dietary folate has an important role in the formation of SAM, and can be a limiting factor in the associated pathway. In the folate cycle (Figure 1), folate is imported into cells and metabolized into its active form

305 THF, which is converted to 5,10-methyl-THF by hydroxymethyl transferase (vitamin B-
306 6 as cofactor). It is then reduced to 5-methyl-THF by MTHFR (riboflavin as cofactor),
307 and to complete the folate cycle, 5-methyl-THF is demethylated to form THF. With the
308 demethylation of 5-methyl-THF, the methyl group is donated into the Met cycle through
309 the methylation of homocysteine by Met synthase and its cofactor vitamin B-12 (42, 43,
310 46, 47).

311 The ability of folate to lower homocysteine levels indirectly suggests it might
312 have a positive influence on CVD, considering that high homocysteine plasma levels
313 are linked to cardiovascular mortality in CKD (7). Accordingly, HD patients display
314 low folic acid intake, low folate serum levels and high homocysteine levels (44, 48).
315 Thus, folic acid therapy may be an important factor for these patients. Indeed, in a study
316 targeting the high homocysteine levels in CKD, folate supplementation reduced but did
317 not normalize plasma homocysteine levels (45, 49). However, other studies have
318 reported no consistent effect of extended supplementation with folic acid on
319 homocysteine levels in CVD and CKD cohorts (46, 47, 50, 51). An insight into these
320 equivocal reports can be gained from Xiao et al. (19) who have discussed how elevated
321 plasma homocysteine levels are associated with an increased risk of CVD, and why
322 some intervention studies with vitamin B and folic acid supplementation are not able to
323 reduce its levels. One possibility is that homocysteine is simply a marker of increased
324 CVD risk and that SAH accumulation may be the cause of increased risk. Studies on
325 atherosclerosis, CKD, diabetes and obesity have all shown that SAH levels better reflect
326 an increased cardiovascular risk than homocysteine (48, 49, 52, 53). One proposed
327 mechanism is that SAH promotes apoptosis of endothelial cells, independently
328 of homocysteine levels, and enhances Dihydronicotinamide-adenine dinucleotide
329 phosphate (NADPH) oxidase expression, increasing the production of reactive oxygen

species (50, 54). Furthermore, SAH is a powerful competitive inhibitor of SAM, which is also increased, both intra- and extracellularly, in various pre-clinical models of hyperhomocysteinaemia, including uremia (51,55). As a result, several methyl transfer reactions may be impaired, suggesting that methylation biochemistry is imbalanced (52, 56). High levels of homocysteine and SAH can then be associated with altered DNA methylation profiles in CKD (7). It is worth mentioning, however, that while homocysteine levels are linked to DNA methylation profiles (53, 57), these may be specific to subsets of genetic elements and not to the DNA global methylation levels. Global DNA hypomethylation is more typically observed in most non-communicable and age related diseases. It is notable that confounding factors, such as inflammation, oxidative stress, dyslipidemia and folate supplementation, may explain the lack of agreement in reports from disparate clinical studies (52, 53, 56, 57).

2.3 Vitamin B-12

Vitamin B-12, a water-soluble vitamin, is one of eight B vitamins naturally present in animal and dairy products, and generally not present in plant foods. (Table 1) (54, 58). Vitamin B-12, together with folate, plays a key role in the formation of SAM in one-carbon metabolism (Figure 1) and works as a coenzyme for Met synthesis via its action in the transfer of the methyl group from 5-methyl-THF to homocysteine to form Met (55, 59). Vitamin B-12 deficiency is associated with anemia and neurological disorders., Additionally, low vitamin B-12 is associated with higher plasma homocysteine, a risk factor for CVD (60). Impairment of its conversion to Met leads to DNA hypomethylation (56, 61). a key feature of normal ageing processes.

Correspondingly, CKD patients also have high prevalence of vitamin B-12 deficiency, in keeping with it being a disease of accelerated ageing (62). Furthemore, in

an observational cohort study, it has been shown that vitamin B-12 is not associated with albuminuria or reduced kidney function in both a univariate or multivariable-adjusted models. However, in patients with elevated homocysteine levels, higher vitamin B-12 concentrations were associated with an increased prevalence of reduced kidney function. The combination of elevated homocysteine along with increased B-12 suggests the possibility of a resistance to the usual effects of vitamin B-12 in these individuals (63). Moreover, Soohoo et al. (64) observed association between high vitamin B-12 levels and mortality in HD patients. In patients with higher predisposition to inflammation, such as the HD population, decreased production of transcobalamin II may lead to reduced uptake of circulating vitamin B-12 by peripheral tissues, and heightened synthesis of transcobalamins I and III further augment accumulation of B-12 in serum (64).

The ratio of folic acid and vitamin B-12 may play an important role in determining global DNA methylation levels. Indeed, it has been reported, that a vitamin B-12 deficient diet, although with maternal folic acid supplementation, reduced total global DNA methylation levels in rats (57, 65). In human studies, children of mothers who had lower vitamin B-12 intake and high folate concentrations, presented insulin resistance and adiposity, suggesting that defects in one-carbon metabolism might be fundamental to intrauterine programming of adult disease (58, 66).

2.4 Choline

Choline, a natural amine recognized as an essential nutrient, is widely distributed in foods, mostly in the form of phosphatidylcholine in the cell membranes (54, 58). Although choline can be synthesized in the liver, via the sequential methylation of phosphatidylethanolamine, the amount that the body naturally synthesizes is not

sufficient to meet human requirements (59, 67). Current evidence suggests that nearly 90% of adults do not achieve the recommended daily adequate intake of choline (425 and 550 mg/day for women and men, respectively) (61, 68). Choline deficiency leads to increased plasma homocysteine levels (thus there is a diminished capacity to methylate homocysteine to form Met), which is associated with CVD and cognitive decline (62,69) among other diseases, as well as genomic DNA hypomethylation through reduced tissue levels of SAM (63, 70).

Under physiological conditions choline is excreted through urine. However, in CKD patients this methyl donor is accumulated. In dialysis patients, choline is cleared and plasma free choline concentration falls during hemodialysis, but returns to baseline levels 6 hrs later (71). Epidemiological studies have found that a high blood choline level is positively associated with metabolic syndrome (or dyslipidemia) (72) and major adverse cardiovascular events (73, 74). It is unclear whether choline itself, or metabolites like trimethylamine-N-oxide (TMAO), the production of which initially requires the metabolic activities of gut microbiota to generate it, contributes to the adverse events experienced by individuals with high choline intake (75). Indeed, several human studies have associated high levels of TMAO to CVD (76, 77, 78 ,79, 80). Moreover, a direct inverse relationship between TMAO levels and renal function has been observed, with a severe elevation of TMAO seen in advanced CKD (77,81). Coinciding with this, TMAO has widely been identified as a promoter of atherosclerosis (70, 82), and accelerated atherosclerosis is exacerbated in patients suffering from CKD compared to those with normal renal function.

2.5 Betaine

Betaine (glycine betaine, N,N,N-trimethylglycine) is an important non-essential nutrient derived from either dietary intake, or via choline oxidation. The latter involves choline oxidation to betaine aldehyde in the inner mitochondrial membrane, and then to betaine; both oxidation steps are catalyzed by CHDH. This reaction is essentially irreversible, as betaine cannot be reduced back to choline (71, 83). Betaine is found in a variety of food sources (Table 1) and its main physiological role is as a methyl donor, playing an essential role in the transition from homocysteine to Met, catalyzed by BHMT. Thus, betaine regulates the concentrations of SAH and is essential for one-carbon metabolism (72, 73, 74, 84).

Just like choline, betaine is a TMA-containing nutrient, that leads to the production of TMAO (74, 81). This uremic toxin is elevated in CKD, and is associated with high risks of progressive renal fibrosis, thereby significantly increasing mortality rates from the disease (75, 85).

3. The microbiome - linking “inflammaging” to the epigenome

A growing body of evidence has indicated that social, psychological life style and nutritional risk factors influence the trajectory of age related health and age related morbidities by acting either independently, cumulatively, or synergistically with an individual’s genetics, and in particular epigenetics, thus determining health span (3). Accelerated biological ageing (i.e. ‘miles on the clock’) is also a feature of age related morbidities, where disease specific processes are layered upon dysregulated ageing processes. This thesis has been extensively exemplified for the kidney, where CKD has been classified as a condition of accelerated ageing (5) + Stenvinkel P, Larsson T. Chronic kidney disease – a model of premature aging. Am J Kidney Dis. 2013 Aug;62(2):339-51.

Mechanistic insight into how dietary and epigenetic factors regulate ageing throughout the life-course, linked to a decline in renal function with ageing, is already proving of significant value (34). Recently, evidence has emerged indicating that (i) epigenetic regulation of nutrient sensing pathways and (ii) nutritional differences tied to socioeconomic position, can differentially affect the renal ageing process in particular age-related genomic hypomethylation and inflammatory status. In both instances, renal function reflected changes in ageing processes and their associated epigenetic regulation (3, 34).

An outstanding problem, however, remains identifying factors driving "inflammaging". Intuitively, the burden of aged (senescent) cells generates a pro-inflammatory environment via a senescence associated secretory phenotype (SASP), that poison the surrounding tissue. However, in epidemiological cohorts, <15% of the level of systemic inflammation can be explained on the basis of cellular aging.

A key component of the inflammatory burden of ageing may be provided by the microbiome. The human microbiome refers to the entire collection of genetic material belonging to the microorganisms residing within the human body, including bacteria, archaea, fungi, viruses, helminths and protozoa (76, 86). The microbiome is an integral part of the normal host function, and a mutual relationship exists between the human body and its associated microbiome (77, 87). A particular element of the microbiomes function, which is crucial to its host is its ability to provide a means of metabolising otherwise inaccessible nutrients needed for example, for the production of short chain fatty acids during energy metabolism (78, 88). The microbiome also produce metabolites, which are necessary for amino acid production essential to the host, and in turn is associated with maintaining our epigenetic landscape (3, 4).

As the gut microbiome changes with both chronological and biological age (79, 89), one novel hypothesis that has gained much attraction is that the microbial metabolite TMAO is central to the inter-relationship between “inflammaging”, health span and the age-related epigenome. This pro-atherogenic and pro-inflammatory compound is derived from microbial metabolism of phosphatidylcholine, L-carnitine and lecithin, which are found in red meat, fish and eggs, so providing a mechanistic link between nutrition, ageing and the epigenome (3, 35). Production of TMA, the TMAO precursor, was also observed to be greater in frail older people that consumed a restricted diet than healthy older people, in a manner that could be linked to differences in their microbiome coding capacity (80, 90). There is also a further emerging role for the microbiome in epigenetics through production of butyrate that inhibits histone deacetylases (81, 91).

In CKD, microbial dysbiosis correlates with altered metabolism of proteins and amino acids leading to the production of toxic compounds including ammonia, phenols, indoles and most notably TMAO (2, 35). A study into the intestinal microbial populations of Chinese CKD patients found eight genes associated with TMAO production. One of these genes was associated with betaine metabolism and showed reduced expression in CKD. This suggests that gut dysbiosis leads to abnormal betaine metabolism, thus producing a redundant level of TMA free to be oxidised to toxic TMAO (91). Thus, monitoring the dietary intake of TMA producing nutrients, such as betaine, choline and L-carnitine, along with the identification of key members of the gut microbiota associated with their metabolism offer potential therapeutic strategies to alleviate the burden of renal disease.

In addition, the loss of symbiosis in the gut also contributes to impaired intestinal epithelial barrier function leading to translocation of bacterial-derived uremic

toxins into the systemic circulation (92). This contribute to inflammation, protein-energy wasting, insulin resistance, and exacerbation of the risk of CVD during CKD progression (93). With these links between nutrition, microbial metabolism and epigenetic regulation in mind, studying the interplay between these key factors are important to further investigations into CKD progression, and could offer a potential therapeutic target.

4. Conclusions

Nutrient intake has a direct effect on the epigenome and emerging evidence is highlighting a complex interplay with the microbiome. This is both pertinent to age-related health and inflammation and involves modulation of one-carbon metabolism. This aspect of cellular biochemistry is of interest in CKD, as the exposure to a physiologically aberrant uremic milieu is a potential factor in the promotion of dysregulation of the epigenetic landscape. Additionally, any nutritional impact on the microbiome and its interaction with host physiology will again directly affect the epigenome. Alteration of the microbiome is a known feature of both CKD and "inflammaging". It is intuitive to consider the modulation of the microbiome, either by variation in diet, or by restoration of microbial diversity in the gut using live bio-therapeutics (i.e. implantation of hub microbes to alter diversity), to mitigate loss of diversity with ageing or in accelerated ageing linked to morbidities, such as CKD. Studies evaluating the effects of dietary supplementation, such as methyl donor nutrients, on the epigenome of CKD and gut microbiota metabolism, remain limited. Future studies are merited as a link is intuitive and may easily achieve direct clinical benefit. One further benefit from such approaches may be a reduction of age or disease

related inflammatory burden. This is again easily investigated, achievable and merits action.

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Figure 1. Methylation playing a crucial role in gene expression

DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group (CH3) to the 5-position of a cytosine, generating 5-methylcytosine. This chemical modification inhibits gene expression when areas rich in CpG (CpG Island) are present within promoter regions.

Figure 2. Dietary nutrients and one-carbon metabolism

One-carbon metabolism comprises the folate cycle and the methionine cycle that are interconnected in a complex process involving dietary nutrients as substrates or enzymatic cofactors. These nutrients and their respective dietary sources are depicted above. Dietary folate is metabolized into THF, which is converted to 5,10-methyl THF and finally to 5-methyl THF, which is demethylated and donates the methyl group into the methionine cycle through the methylation of homocysteine by a methionine synthase B-12-dependent reaction. Homocysteine can also receive a methyl group from betaine to form methionine. Methionine is then converted to SAM, the universal methyl donor required for DNA methylation. After the methyl group is transferred from SAM to the DNA molecule, forming 5-Methylcitosyne, SAH is formed and hydrolyzed to homocysteine, which returns to methionine cycle.

BHMT: betaine homocysteine methyltransferase; DNMT: DNA methyltransferase; MTHFR: methylenetetrahydrofolate reductase; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SHMT: serine hydroxymethyl transferase; THF: form tetrahydrofolate.

	Source Food	Forms	Functions
Methionine	**(g/100 g)** Brazil nut 1.12 Chicken 0.79 Beef and pork 0.77 Cheese 0.52 Eggs 0.40	L- Methionine	Central molecule in one-carbon metabolism Precursor to S-adenosylmethionine Influences maximal lifespan in mammals Normal growth and development
Folate (Vitamin B-9)	**(µg/100 g)** Spinach 199 Beans 149 Broccoli 108 Avocado 89 Beets 80 Eggs 51	Folic acid Tetrahydrofolic acid, Methyltetrahydrofolate Methenyltetrahydrofolate Folinic acid	Formation of S-adenosylmethionine May lower homocysteine levels
Vitamin B-12	**(µg/100 g)** Fish 6.22 Beef 1.68 Eggs 0.89 Yogurt 0.52 Poultry 0.36	Methyl cobalamin 5-deoxyadenosylcobalamin	Formation of S-adenosylmethionine Red blood cell formation, neurological function and DNA synthesis
Choline	**(mg/100 g)** Egg yolk 689 Liver 330 Wheat germ 180 Soybeans 120 Meat 100 Salmon 79	Phosphatidylcholine, Lecithin Choline bitartrate Choline choride	Phosphatidylcholine syntheses (vital phospholipid for cell membranes) Precursor for the neurotransmitter acetylcholine (central role in brain development) Oxidized, in the liver and kidney, or metabolized by gut bacteria to betaine (indirect methyl group donor for one-carbon metabolism)
Betaine	**(mg/100 g)** Quinoa 630 Wheat germ 410 Bran 320 Spaghetti 140 Beets 130 Spinach 120 Seafood 23	Betaine HCl	Essential for one-carbon metabolism

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