Uraemic solutes as therapeutic targets in CKD-associated cardiovascular disease

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Abstract | Chronic kidney disease (CKD) is characterized by the retention of a myriad of solutes termed uraemic (or uremic) toxins, which inflict damage to several organs, including the cardiovascular system. Uraemic toxins can induce hallmarks of cardiovascular disease (CVD), such as atherothrombosis, heart failure, dysrhythmias, vessel calcification and dysregulated angiogenesis. CVD is an important driver of mortality in patients with CKD; however, reliance on conventional approaches to managing CVD risk is insufficient in these patients, underscoring a need to target risk factors that are specific to CKD. Mounting evidence suggests that targeting uraemic toxins and/or pathways induced by uraemic toxins, including tryptophan metabolites and trimethylamine *N*-oxide (TMAO), can lower the risk of CVD in patients with CKD. Although tangible therapies resulting from our growing knowledge of uraemic toxicity are yet to materialize, a number of pharmacological and non-pharmacological approaches have the potential to abrogate the effects of uraemic toxins, for example, by decreasing the production of uraemic toxins, by modifying metabolic pathways induced by uraemic toxins such as those controlled by aryl hydrocarbon receptor signalling and by augmenting the clearance of uraemic toxins.

Chronic kidney disease (CKD) is a major public health concern, estimated to affect 13% of the global population[1]. In the USA, approximately 30 million people, or one in seven individuals, have CKD, and this number is predicted to increase[2]. Globally, CKD results in 5–10 million deaths annually, a figure that is likely to be an underestimate[3,4]. In the USA, 340 people initiate dialysis treatment every 24 h (REF.[2]) and 240 patients on dialysis die daily[5]. Mortality among patients with CKD is largely attributable to an enormous cardiovascular disease (CVD) burden and an increased risk of major adverse cardiovascular events, including atherothrombotic disorders that drive myocardial infarction and stroke, heart failure, arrhythmias and sudden cardiac deaths in both adult and paediatric populations[6–10]. These conditions contribute to a CVD mortality in patients with CKD that is at least double that of CVD in patients without CKD[11,12]. CVD risk also increases with severity of CKD: 27.5% of deaths among patients with early-stage CKD (stages 1–2) can be attributed to CVD, increasing to 58% among patients with advanced-stage CKD (stages 4–5). In fact, the life expectancy of patients with advanced CKD is shortened by over 16 years owing to CVD-related mortality[13,14].

The previously held dogma that CVD and CKD are merely associated entities resulting from the existence of common risk factors has been debunked by large epidemiological studies[13]. A meta-analysis of 1.4 million individuals from 30 published cohort studies suggested that after adjusting for traditional cardiovascular risk factors (such as age, sex, ethnicity, hypertension, diabetes status, high cholesterol and smoking), the risk gradient for cardiovascular mortality was evident at early stages of CKD and increased linearly with progression of CKD and albuminuria[11,12]. Another meta-analysis that involved 1.02 million participants from 30 general population and high-risk cardiovascular cohorts and 13 CKD cohorts found that the association of CKD with cardiovascular mortality was not influenced by the presence or absence of diabetes or hypertension[15]. This conclusion was corroborated by findings from the Alberta Kidney Disease Network of 1.26 million participants, which demonstrated that the rate of incident myocardial infarction was substantially lower among patients with diabetes mellitus and normal kidney function than among those with concomitant CKD and proteinuria[16].

Further supporting evidence that CKD is an independent risk factor for CVD stems from the observation that interventions that target conventional CVD risk factors do not adequately prevent cardiovascular events in patients with CKD[17–19]. Large clinical trials have demonstrated that statins have limited ability to reduce primary

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Key points

- Patients with chronic kidney disease (CKD) retain myriad chemical compounds, known as uraemic toxins, that mediate systemic complications including cardiovascular disease (CVD); levels of these toxins rise with CKD progression, further increasing cardiovascular risk.
- Early interventions that target conventional cardiovascular risk factors, such as obesity and hypertension, combined with approaches to directly target uraemic toxins have potential to lower the risk of CVD in patients with CKD.
- Non-pharmacological measures to target uraemic toxins include approaches to reduce their biosynthesis through dietary interventions and/or microbial manipulation; both of these approaches have limitations.
- Pharmacological strategies to suppress cellular events triggered by uraemic toxins are rapidly emerging as an attractive approach and include inhibitors of the aryl hydrocarbon receptor pathway, kinase inhibitors, Klotho or kynureninase supplementation, AST-120, meldonium and 3,3-dimethyl-1-butanol (DMB).

cardiovascular events in patients with CKD[17,18]. Similar results have been noted for other cardiovascular protective agents[19]. Collectively, these studies indicate that CKD is a strong and independent risk factor for CVD.

A growing body of evidence has linked the cardiovascular risk of CKD to an accumulation of uraemic toxins that occurs with progression of CKD. For patients with kidney failure, kidney transplantation represents the optimal form of kidney replacement therapy in terms of survival outcomes[20–22]. Kidney transplantation is also associated with a reduced risk of cardiovascular events compared with haemodialysis[23,24], and in fact, has been reported to reverse end-organ toxicities, such as cardiac fibrosis[25], arterial stiffness[26] and improve remodelling and cardiovascular reserve[27]. Importantly, and unlike haemodialysis, kidney transplantation is associated with a significant reduction in the levels of several uraemic toxins[28–30], which is likely to underlie some of the benefits observed with transplantation. The identification of uraemic toxins as CKD-specific risk factors for CVD renders it imperative to consider strategies to lower their levels or abrogate their toxicity. This Review summarizes pharmacological and non-pharmacological means of abrogating the effects of uraemic toxins. Device therapies, including innovations in dialysis, have been reviewed elsewhere[31,32] and are not covered here.

CKD: a unique milieu for CVD

CKD is characterized by the retention of several toxic metabolites and solutes that are appropriately termed uraemic toxins. These toxins are chemicals that accumulate as a result of failed elimination by the kidneys, and through interaction with biological processes produce a response that is unfavourable to the host[33]. Based on physicochemical characteristics that determine their response to conventional haemodialysis, uraemic toxins are traditionally classified as either free water-soluble low-molecular-weight solutes, protein-bound (PB) uraemic toxins and middle molecules. Low-molecular-weight solutes include phosphorus, urea and creatinine; middle molecules include fibroblast growth factor 23 (FGF23) and β_2-microglobulin; whereas PB uraemic toxins include indoxyl sulfate, indoxyl acetate, kynurenine, kynurenic acid, *p*-cresyl sulfate[33–35] and indole-3 acetic acid. Trimethylamine *N*-oxide (TMAO) is another uraemic toxin that has been linked to CVD in the general population and is also found at elevated levels in patients with CKD[36]. The association of uraemic toxins such as phosphorus and FGF23 with CVD has been reviewed elsewhere[37,38]; here, we focus on PB uraemic toxins.

Generation and metabolism of PB uraemic toxins

Many uraemic toxins are the products of dietary constituents. For example, *p*-cresyl sulfate is derived from tyrosine, and indoxyl sulfate, indoxyl acetate, kynurenine and kynurenic acid are products of tryptophan metabolism (FIG. 1a). Dietary tryptophan is catabolized through distinct pathways. A small fraction of tryptophan undergoes degradation in the intestine by tryptophanase-containing bacteria to generate indole. Upon absorption through the portal circulation, indole is then converted to indoxyl and then transformed into indoxyl sulfate and indoxyl acetate for excretion through the kidneys[39]. A large proportion (>90%) of dietary tryptophan is absorbed directly through the portal circulation and is subsequently converted to *N*-formyl-L-kynurenine by tryptophan 2,3-dioxygenase 2 (TDO2) in the liver and indoleamine 2,3-dioxygenase 1 (IDO1) and IDO2 in the peripheral tissues to form kynurenine[40] (FIG. 1b). Kynurenine is further catabolized by kynureninase to increase subsequent downstream products such as anthranilic acid and quinolinic acid, which represents the major route of kynurenine clearance[41,42], or by kynurenine amino transferases to kynurenic acid[43]. In kidney failure, an accumulation of tryptophan metabolites occurs as a consequence of its increased production in the gut owing to gut dysbiosis, alterations in enzyme activity and its ineffective excretion by the kidneys[39,44–46]. In contrast to these PB uraemic toxins, TMAO is a low-molecular-weight metabolic product of dietary precursors L-carnitine, choline and phosphatidylcholine, which are derived from red meat, eggs, fish and poultry (FIG. 2). The intestinal microbiome degrades these precursors into trimethylamine (TMA), which is further converted into TMAO in the liver[36,47]. Elevated TMAO levels in CKD are likely to be due to its potentially enhanced production and reduced filtration[48].

Cardiovascular effects of PB uraemic toxins

A wealth of clinical studies has linked the above-described uraemic toxins to cardiovascular mortality in patients with CKD[49–53], in association with profound cardiac and vascular dysfunction. Although some phenotypic overlap exists in the profile of CVD among the general population and patients with CKD, distinct differences exist[54] imparted by the specific cardiac and vascular toxicity of TMAO[36,53,55,56], indoxyl sulfate[57,58], *p*-cresyl sulfate[51,59], kynurenine[60] and indole-3 acetic acid[61], as reviewed elsewhere[60].

Cardiac dysfunction induced by uraemic toxins is characterized pathologically by myocardial fibrosis and remodelling abnormalities[62] (FIG. 3). Although the pathogenesis underling these entities is likely to be multifactorial, certain uraemic toxins are implicated. For example, treatment of cultured neonatal rat cardiomyocytes with indoxyl sulfate induces protein

synthesis and increases cell volume, reminiscent of the cardiac hypertrophy observed in patients with CKD[63]. Rats treated with indoxyl sulfate also showed increased myocardial fibrosis[64]. Administration of *p*-cresyl sulfate to mice induced cardiomyocyte apoptosis — an effect that might contribute to diastolic dysfunction in patients with CKD[65]. Mechanisms such as endoplasmic reticulum and oxidative stress are also likely to contribute to myocardial damage induced by uraemic toxins[66,67].

Uraemic vascular disease is characterized by endothelial dysfunction, accelerated atherosclerosis, neointimal hyperplasia, a hyperthrombotic state, abnormal vascular

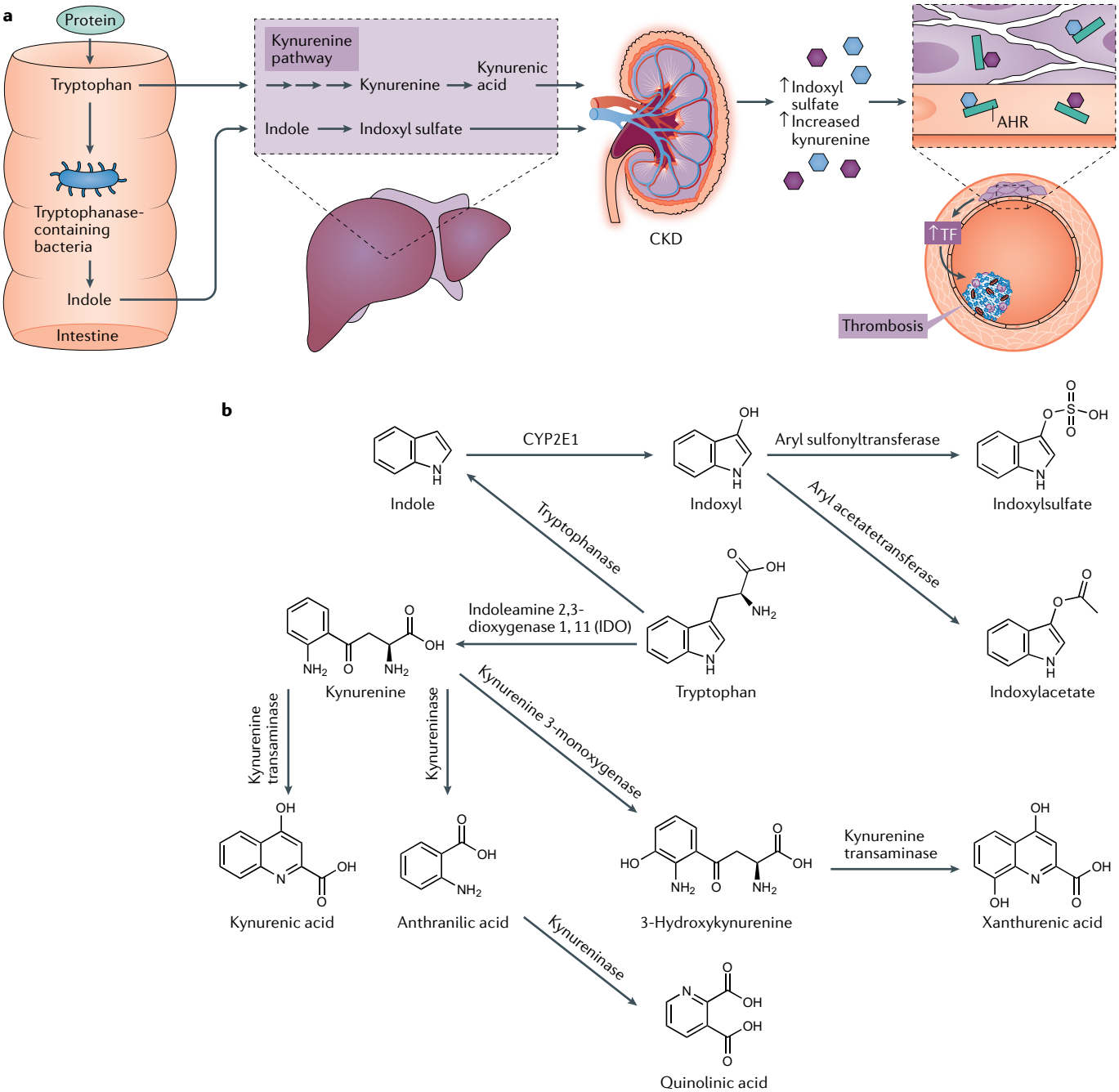

Fig. 1 | **Uraemic toxin production from tryptophan metabolism.** **a** | Tryptophan obtained through dietary sources can be degraded in the intestine by tryptophanase-containing bacteria to produce indole, which is then converted into indolic solutes such as indoxyl sulfate in the liver. Tryptophan absorbed through the portal circulation can also undergo metabolism in the liver to produce kynurenine. Indoxyl sulfate and kynurenine are considered to be uraemic toxins; they are normally excreted by the kidney but are retained in patients with chronic kidney disease (CKD). Elevated levels of these uraemic toxins in patients with CKD activate the aryl hydrocarbon receptor (AHR) to increase levels of tissue factor (TF) in the vessel wall, thereby enhancing thrombotic processes. **b** | A number of uraemic toxins, including indoxyl sulfate, indoxyl acetate, kynurenine and kynurenic acid are derived from tryptophan metabolism. Kynureninase is likely to augment the degradation of kynurenine and increase its downstream metabolites such as anthranilic acid and quinolinic acid, which are also considered to be uraemic toxins. Part **a** adapted with permission from REF.[77], American Society of Nephrology.

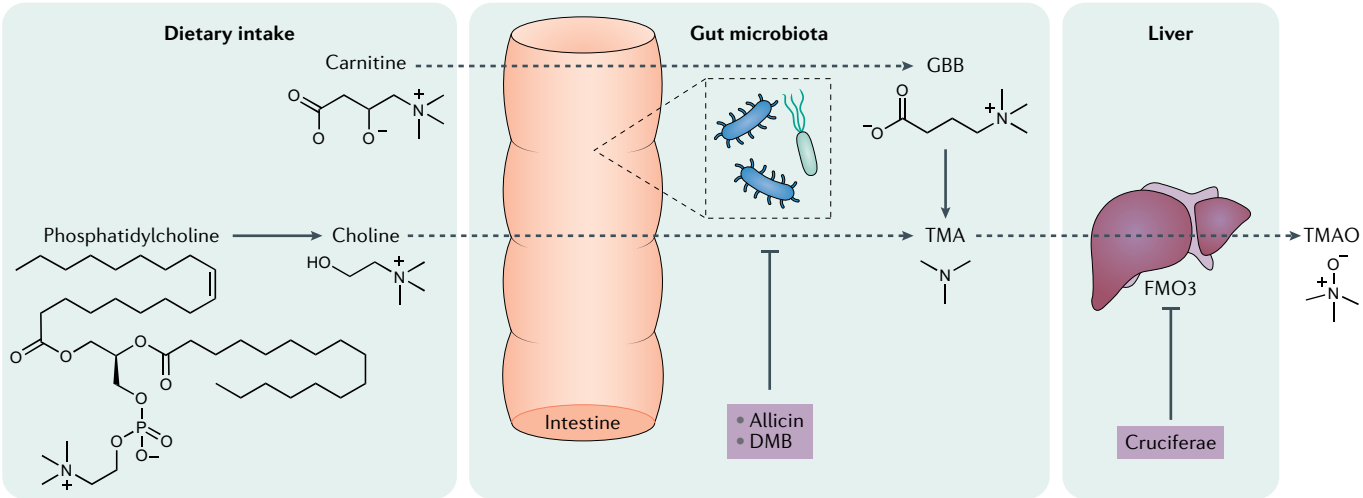

Fig. 2 | **TMAO synthesis.** Trimethylamine *N*-oxide (TMAO) is a multistep process and includes dietary ingredients such as choline and carnitine, which undergoes microbial processing. The intermediate γ-butyrobetaine (GBB) is rapidly converted to trimethylamine (TMA), which is then converted to TMAO, which is normally excreted via the kidneys. Allicin and 3,3-dimethyl-1-butanol (DMB) inhibit the microbiome processing and reduce the circulating levels of TMAO. A vegetarian diet supplemented with members of the Cruciferae family can reduce the activity of FMO3 — a flavin-dependent monooxygenase enzyme that is responsible for TMAO synthesis — and can therefore reduce the production of TMA from L-carnitine.

calcification, microvasculature rarefaction and suppressed angiogenesis (FIG. 3). Uraemic toxins contribute to these pathological conditions by promoting the dysfunction of several cell types including endothelial cells, endothelial progenitor cells, vascular smooth muscle cells (VSMCs), platelets and polymorphonuclear cells. For example, indolic solutes and other uraemic toxins compromise various properties of endothelial cells, including their proliferation, survival, migration, permeability, anticoagulant properties and nitric oxide-induced vasodilation[68–71]. Of note, aryl hydrocarbon receptor (AHR) signalling[72–75] may be particularly important for the cardiovascular effects of uraemic toxins. In endothelial cells and VSMCs, indoxyl sulfate and kynurenine bind to the ligand-binding domain of AHR, leading to AHR activation and inducing the transcription of *F3*, which encodes tissue factor[74] (FIG. 4). Tissue factor is the primary trigger of the extrinsic coagulation cascade and drives thrombosis after vascular injury (for example, after vascular surgery or endovascular procedures). In addition to increasing *F3* transcription, ligand-activated AHR binds tissue factor in the cytosol to prevent its degradation[73,76]. The association of this indoxyl sulfate and the kynurenine–AHR–tissue factor axis with thrombosis has been validated in two independent patient cohorts[77]. Moreover, AHR activation is also implicated in the atherosclerosis of *Apoe*-knockout mice[78]. Uraemic toxins may also affect thrombotic processes via actions on endothelial cells and VSMCs. VSMC proliferation is a hallmark of intimal stenosis and VSMCs are a major contributor to thrombosis in arterial diseases. Several uraemic toxins, such as inorganic phosphate and indoxyl sulfate, induce VSMC proliferation, migration and calcification[79] and augment the prothrombotic properties of VSMCs[72,73,76]. In addition, the CKD milieu alters platelet functions, which can contribute to atherothrombosis[19,54,80,81].

The profound adverse effects of uraemic toxins on cardiovascular biology[82] indicates that therapeutic targeting of uraemic toxins or their downstream mechanisms could lead to cardiovascular benefits for patients with CKD. As described in the following sections, potential therapeutic approaches could involve non-pharmacological or pharmacological interventions. Given the complex biology of uraemic toxins, a multimodal approach may be warranted to effectively control their protean effects on the cardiovascular system.

Non-pharmacological interventions

The goal of non-pharmacological interventions is to modulate the production of and/or improve the clearance of uraemic toxins. The rationale for these interventions is built on the understanding that some uraemic toxins are derived from specific dietary constituents such as amino acids (for example, tryptophan for indoxyl sulfate, kynurenine and indoxyl acetate; tyrosine for *p*-cresyl sulfate), fatty acids or other nutrients such as choline (for TMAO) that are subsequently processed by the intestinal microbiome (FIGS 1,2). The intermediate metabolic products that arise from this processing are absorbed into the portal circulation and undergo further biotransformation in the liver through phase I metabolism and phase II metabolism. In blood, some uraemic toxins bind tightly to albumin[83] and are excreted in urine through tubular secretion[84,85]. Uraemic toxins are pathologically elevated even in patients with early-stage CKD, and continue to rise with disease progression[72,86,87]. However, the importance of uraemic toxin biogenesis and clearance to total uraemic toxin level is underscored by the fact that concentrations of uraemic toxins vary substantially between patients at the same CKD stage[72,77,86–88]. Indeed, uraemic toxins are influenced not only by their excretion via the kidneys but also by dietary intake, processing by

Phase I metabolism
Biochemical processing of the parent drug (by oxidation, reduction or hydrolysis) to convert it into a more polar molecule.

Phase II metabolism
A phase of drug metabolism that involves conjugation of the drug by coupling it or its metabolites to another molecule to augment its excretion.

Transamination
A biochemical process whereby an amino group from an amino acid is exchanged for a keto acid, generating an amino acid version of the keto acid and a keto acid version of the original amino acid.

the intestinal microbiome and biotransformation in the liver[39,40,89–92], all of which can vary between individuals and may be considered as targets for intervention.

Nutritional interventions

Dietary modification has long been a cornerstone in the management of patients with CKD. For example, restriction of dietary components such as proteins reduces substrates for uraemic toxin generation, and in principle reduces uraemic manifestations[93,94]. Over time, this approach tempers the emergence of metabolic abnormalities and potentially delays the need for dialysis[95]; however, the optimal amount of protein intake for patients with CKD and kidney failure remains a topic of debate[96].

Several studies have examined the effects of low protein, vegetarian or Mediterranean diets on CKD progression, but little is known about the influence of these diets on CVD events. One study of 29 healthy individuals given a high protein diet (that is, a target protein intake of >25% of total energy intake) or low protein diet (that is, a target protein intake of <25% of total energy intake) for 2 weeks reported that individuals on a low protein diet demonstrated a reduction in serum levels of indoxyl sulfate, indoxyl glucuronide, kynurenine and quinolinic acid, and reduced urinary excretion of indoxyl sulfate — demonstrating the feasibility of this approach to lowering uraemic toxin levels in patients with CKD[97]. A meta-analysis of 16 controlled clinical trials, each involving at least 30 patients with CKD revealed that dietary restriction of protein intake to low or very low levels (<0.8 g/kg per day or <0.4 g/kg per day, respectively), for a time period of 6–36 months, improved levels of bicarbonate, phosphorus and blood urea nitrogen, slowed the rate of progression to kidney failure, and displayed a trend towards lower rates of all-cause mortality. Furthermore, patients on very low protein diets had slower progression of kidney failure than patients on a low protein diet[98]. By contrast, the Modification of Diet in Renal Disease (MDRD) study found no beneficial effects of reducing protein intake on the progression of kidney failure, and noted a signal for malnutrition and a negative impact on survival[99,100]. Of note, none of these studies examined the effect of protein restriction on CVD events or CVD-related mortality.

Although the optimal level of protein restriction required to ameliorate uraemic toxicity in patients with CKD is unclear, several studies have attempted to compensate for the lack of essential amino acids in a low protein diet by supplementation with ketoanalogues. The rationale for this approach is that α-ketoanalogues should convert into essential amino acids in the body via transamination and thereby compensate for the lack of dietary amino acids obtained through a low protein diet. One meta-analysis that included data from 951 patients demonstrated a benefit of this approach in terms of slowing progression of CKD without causing malnutrition. However, the effect of this intervention on patient survival or CVD mortality benefit was not evaluated[101].

Vegetarian diets may provide different proteins and lower total protein levels than animal-based diets,

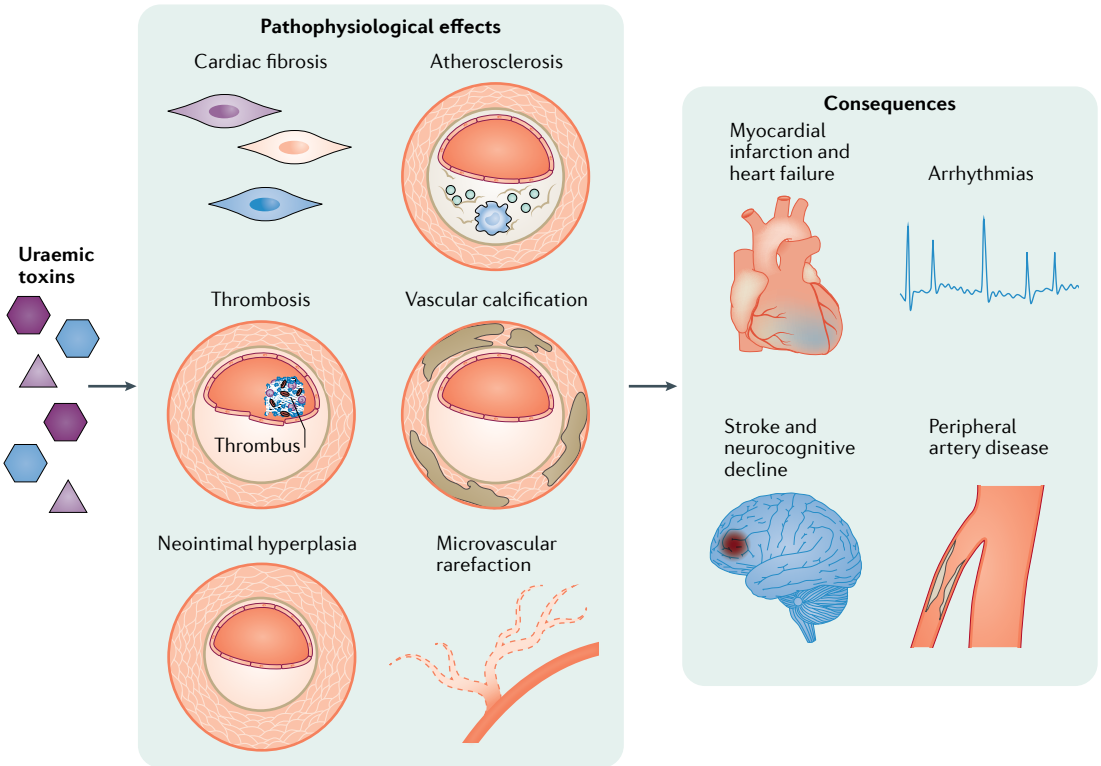

Fig. 3 | **Cardiovascular consequences of uraemic toxicity.** Uraemic toxins can induce a plethora of cardiac and vascular pathological conditions, including cardiac fibrosis, accelerated atherosclerosis, thrombosis, medial vascular calcification and microvascular rarefaction. These processes can, in turn, drive cardiovascular complications such as myocardial infarction, heart failure, arrhythmias, stroke, cognitive decline and peripheral artery disease.

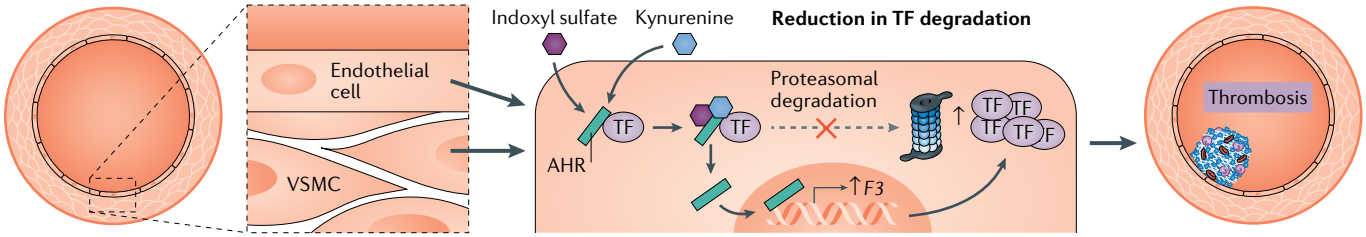

Fig. 4 | **Consequences of AHR activation.** The aryl hydrocarbon receptor (AHR) is a ubiquitously expressed cytosolic protein that undergoes nuclear translocation upon binding with ligands such as indoxyl sulfate and kynurenine. In the nucleus, AHR induces transcription of *F3*, which encodes tissue factor (TF). In the cytosol, ligand-activated AHR protects TF from ubiquitination by STUB1 E3 ligases. Both these processes effectively increase the levels of TF in endothelial cells and vascular smooth muscle cells (VSMCs), which, upon rupture of a plaque or vascular intervention, triggers thrombosis through the extrinsic coagulation pathway.

and some evidence suggests that these differences may translate into beneficial effects on cardiovascular indices, inflammatory markers, proteinuria and glomerular filtration rate[102,103]. Vegetarian diets also alter the microbiome in a favourable manner. For example, plant-based diets stimulate the growth of butyrate-producing bacteria and provide fibres that induce the fermentation of short chain fatty acids in the colon, both of which improve epithelial integrity, thereby preventing the release of pro-inflammatory factors from the intestine to the circulation[104,105]. However, one study that examined the effect of the largely plant-based Mediterranean diet on gut-derived uraemic toxins found that this dietary intervention did not lower levels of the studied uraemic toxins or arterial plaque burden — a surrogate of cardiovascular health[106].

Various dietary permutations have also been attempted to reduce levels of TMAO. Studies in rodents and humans have shown that a high fat diet increases postprandial levels of TMAO[107,108]. Reducing the dietary precursors of TMAO through a reduction in dietary levels of red meat, eggs, fish and poultry may be one option for lowering TMAO levels[94]. A vegetarian diet supplemented with members of the Cruciferae family can reduce the activity of FMO3 — a flavin-dependent monooxygenase enzyme that is responsible for TMAO synthesis — and can thus reduce the production of TMA from L-carnitine[109,110]. Targeted dietary manipulations to specifically inhibit other enzymes involved in TMAO synthesis have also been attempted. For example, the Mediterranean diet is thought to derive some of its known health benefits from allicin[111,112], a biochemical found in garlic and virgin olive oil that modulates gut microbiota through its antimicrobial properties to reduce the metabolism of carnitine to TMAO[113,114]. Moreover, 3,3-dimethyl-1-butanol (DMB), a choline analogue found in virgin olive oil and red wine, inhibits microbial TMA lyases and also reduces TMAO production[115,116]. However, a prospective multicentre study in Spain showing a reduction in cardiovascular events among individuals at a high cardiovascular risk following implementation of a Mediterranean diet supplemented with extra-virgin olive oil[111] did not evaluate individuals with CKD.

TMAO synthesis from dietary L-carnitine involves gut microbiota through two sequential gut microbiota-dependent transformations: namely, the rapid generation of the atherogenic intermediate γ-butyrobetaine (GBB) followed by its transformation into TMA. Dietary interventions can lower the concentration of L-carnitine, but can in parallel modify microbiota to reduce TMAO production. The influence of diet on the two-step, gut-dependent process of TMAO generation has been examined in healthy individuals[117]. Ingestion of oral isotope tracers followed by extensive microbial analysis demonstrated that participants who followed a vegetarian or vegan diet had significantly lower TMAO levels than those of individuals on an omnivorous diet. Thus, reduced ingestion of L-carnitine along with alterations in gut microbiota contributes to generally lower TMAO levels among vegans and vegetarians than among meat eaters[117,118].

Some of the above-described studies have lacked information on uraemic toxin levels and their association with CVD events or CVD mortality. However, in the absence of such information, the potential beneficial effect of a particular diet on the cardiovascular health of patients with CKD should be carefully weighed against their putative limitations, such as increases in potassium and oxalate load[94]. Early and protracted dietary intervention may be required to achieve an appreciable reduction in CVD events among patients with CKD. In this case, long-term compliance may become a limiting factor. Despite these limitations, an accumulating body of evidence suggests that plant-based diets offer distinct advantages for the management of CKD, including beneficial effects on protein, carbohydrate, fat and phosphate levels, as well as improvements in acidosis[94]. Pending additional longitudinal, well-powered studies, patient-tailored diets that reduce uraemic toxins and their related CVD outcomes are yet to be established.

Targeting microbiota

Intestinal microbiota have an integral role in the maintenance of intestinal barrier integrity and regulation of host immunity and metabolic processes, and have been linked to hallmarks of CVD, including hypertension[119] and atherosclerosis[120]. The accumulation of uraemic toxins in patients with CKD and consumption of antibiotics, such as macrolides, β-lactams and fluroquinolones, can profoundly affect the composition of intestinal microbiota, resulting in 'uraemic gut dysbiosis'[121]. One mouse model of CKD demonstrated intestinal dysbiosis of various

Cruciferae
A large family of plants with four-petalled flowers that includes cabbage, brussels sprouts, broccoli and turnips.

proteobacteria, increased translocation of bacteria across the intestinal barrier and increased serum levels of bacterial endotoxin[122]. The dysbiosis of patients with CKD is characterized by a decrease in the abundance of Clostridia and *Bacillus* spp.[122]. and an increase in several bacterial families that contain urease (for example, Alteromonadaceae and Cellulomonadaceae), uricase (for example, Cellulomonadaceae and Dermabacteraceae) and tryptophanase (for example, Enterobacteriaceae and Verrucomicrobiaceae)[123]. An analysis of 19 intestinal microbial families that were overexpressed in patients with kidney failure compared with levels in healthy individuals demonstrated that 12 possessed urease, 5 possessed uricase and 4 possessed indole and p-Cresol-forming enzymes[123]. These changes would be expected to augment the production of urea, uric acid and indole, and compromise intestinal barrier function, resulting in the translocation of bacteria and toxins into the circulation[124,125]. A notable depletion of short-chain fatty acids such as butyrate and propionate in patients with CKD indicates a shift away from saccharolytic fermentation (indicative of gut microbiome homeostasis) towards proteolytic fermentation (resulting in the production of harmful uraemic toxins from amino acids and proteins). These perturbations negatively affect the cardiovascular health of patients with CKD, as reviewed elsewhere[126]. Collectively, these findings underlie the rationale to restore the microbiome balance in patients with CKD.

The microbiome can be manipulated using a number of strategies, including ingestion of unprocessed fibre-rich food items, avoidance of certain drugs such as proton pump inhibitors and/or administration of prebiotics (such as arabinoxylan oligosaccharides or non-digestible oligo and polysaccharides), probiotics, or synbiotics, as summarized in detail elsewhere[127]. Human studies of prebiotics in patients with CKD have yielded mixed results[128]. A 12-week study in which patients with CKD were given muffins loaded with pea hull and the prebiotic inulin[128], based largely on the hypothesis that the increased fibre load would aid delivery of the prebiotic to the colon and thereby increase saccharolytic fermentation, found that this intervention reduced levels of *p*-cresyl sulfate in blood. Following the same rationale, administration of an α-glucosidase inhibitor (acarbose)[129] and the sodium–glucose co-transporter 2 inhibitor canagliflozin[130] to block carbohydrate absorption in the small intestine and increase levels of carbohydrates for saccharolytic fermentation in the large colon of mice also reduced serum concentrations of p-Cresol sulfate. That study used mice with normal kidney function and this approach should be applied to a CKD mouse model to examine the effect of this intervention on uraemic toxins.

In animal models of hypertension without CKD, dietary supplementation with the probiotic *Lactobacillus murinus* or use of a high-fibre diet combined with acetate (again, to improve saccharolytic fermentation) reduced blood pressure, cardiac fibrosis and left ventricular hypertrophy[131,132]. Probiotics have also been investigated in patients with CKD. In one 6-week randomized, placebo-controlled, crossover trial, administration of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium longum* to patients with CKD reduced plasma levels of p-Cresol sulfate but not indoxyl sulfate[105]. Studies of synbiotics have shown similarly variable effects on uraemic toxins[133,134]. In one study, administration of a combination of prebiotics (high-molecular-weight inulin, fructo-oligosaccharides and galacto-oligosaccharides) and probiotics (nine different strains across the *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* genera) to patients with non-dialysis CKD for 6 weeks led to a significant reduction in levels of p-Cresol sulfate but not indoxyl sulfate[106]. Except for changes in the stool microbiome (characterized by an increase in *Bifidobacterium* spp. and depletion of Ruminococcaceae spp.), no alterations in parameters such as inflammatory cytokines and oxidative stress biomarkers were noted. Prospective clinical trials are underway to examine the effects of dietary interventions and approaches to manipulating the intestinal microbiota on CKD outcomes[135]; however, these studies are typically short-term and not powered to examine CVD events.

Investigations of the effects of probiotic supplements on TMAO levels have yielded inconsistent results[127,136,137]. One small randomized study found that administration of the multi-strain probiotic VSL#3 did not significantly attenuate the increase in plasma levels of TMAO induced by a 4-week, high-fat diet in non-obese men[137]. Other approaches have involved fecal transplantation or ingestion of genetically engineered bacteria to modulate uraemic toxin-producing enzymes or approaches to scavenge and/or neutralize endotoxins in the circulation as a result of barrier dysfunction caused by gut dysbiosis[138]. Targeting of gut bacteria that harbour the indoxyl sulfate substrate, tryptophanase, or the TMAO substrate, TMA, can lower circulating levels of indoxyl sulfate and TMAO in mice[139,140]. Targeting of the small molecules produced by microbiota (the secretome) has also been suggested as an approach to modulating host–microbiome interactions as a strategy for lowering uraemic toxins[141].

Despite the potential of these approaches to reduce uraemic toxins, the effect of such interventions on CVD risk in patients with CKD remains unclear. Studies involving manipulation of the microbiome have produced conflicting results, potentially because the influence of host factors, such as age, sex, ethnicity, dietary patterns, comorbidities and medication intake, on the microbial landscape remains incompletely defined. Successful translation of microbiome-targeted therapies would also require major obstacles to be addressed, such as the need to maintain a stable microbial ecosystem in the gut of patients despite frequent changes in their environment resulting from exposure to hospital or dialysis units and/or antibiotics, which may threaten the stability of microbiota communities[142]. Most importantly, the ability of these interventions to effectively reduce levels of a range of uraemic toxins over a protracted period of time with resultant clinically meaningful CVD outcomes in patients with CKD is yet to be demonstrated. Marginal reductions in circulating uraemic toxin levels may not be sufficient to derive biological benefits, as demonstrated by a study in which even

Prebiotics
Components of food that induce the growth or activity of beneficial microorganisms to maintain microbial homeostasis.

Probiotics
Live microorganisms that can improve or restore the gut flora to maintain microbial homeostasis.

Synbiotics
A combination of probiotics and prebiotics that are intended to improve the survival and activity of beneficial microorganisms in the gut.

low levels of uraemic toxins were still able to drive prothrombotic processes[72]. Adding to the complexity of this topic, emerging work casts doubt on the contribution of the microbiome to levels of uraemic toxins. A study of 141 patients showed an increase in levels of PB uraemic toxins with CKD progression; however, the levels of these toxins and their precursors remained unchanged in feces[143]. Anaerobic culture of fecal samples from the patients showed no difference in the rate of p-Cresol sulfate, indole and indole-3 acetic acid production, strongly suggesting that increased plasma levels primarily result from the retention of uraemic toxins as a consequence of impaired kidney function rather than an increase in uraemic toxin generation. These results encourage careful reconsideration of alterations in the microbiome as a therapeutic strategy to reduce the burden of uraemic toxins and as such, microbial intervention remains an experimental pre-clinical modality.

Albumin displacers

Human serum albumin (HSA) is the most abundant protein in plasma and the main carrier of PB uraemic toxins[144]. The fatty acid binding sites of HSA serve as ligand binding domains for PB uraemic toxins[145–147], allowing the hydroxyl group of indoxyl sulfate to interact with Tyr410 of HSA while its amide group interacts with Leu430 (REF.[148]) of HSA (FIG. 5a). Indoxyl sulfate also forms salt bridges, hydrogen bounds and van der Wall interactions with other residues in that binding domain[148]. Once bound to HSA, the ability of haemodialysis and haemodiafiltration to clear PB uraemic toxins is limited[149,150]. For example, concentrations of indoxyl sulfate and p-Cresol sulfate are reduced by only 10–35% with haemodialysis, whereas the concentration of more tightly bound uraemic toxins, including 3-carboxy-4-methyl-5-propyl-2-furanproprionic acid, remained unchanged or increased after haemodialysis[151]. Techniques such as fractionated plasma separation and adsorption (so-called albumin dialysis) have been proposed as an approach to clear PB uraemic toxins, but this approach is labour intensive, not easily scalable and fraught with complications such as bleeding and thrombosis[152].

Certain ligands can affect the structure of HSA or alter its affinity to ligands such as indoxyl sulfate[144]. This approach could potentially be used to displace indoxyl sulfate from HSA and increase the amount of HSA-free indoxyl sulfate for clearance by haemodialysis (FIG. 5b). For example, infusion of the competitive ligands ibuprofen and furosemide led to doubling of indoxyl sulfate and indole-3 acetic acid clearance from plasma in an in vitro haemodialysis system[153]. Although an attractive concept, the translation of this technique to the clinical realm is contingent on the identification of an inert and safe displacer, deciphering the off-target effects of the displacer once bound to HSA (for example, effects on hormones and drugs), and the fate of the displacer itself.

Gastrointestinal dialysis

In low-resource settings or situations in which traditional dialysis is not readily available, protocols have been developed for repetitive oral administration of cathartic compounds to promote the excretion of uraemic toxins and excess fluids — methods that are collectively referred to as 'gastrointestinal dialysis'. Although terminated prematurely, an open-label, randomized, controlled trial of oral magnesium oxide (MgO; a cathartic), and the oral carbon adsorbent AST-120 found that MgO, but not AST-120, may be effective in slowing progression of coronary artery calcification in patients with stages 3–4 CKD. However, the MgO group suffered from a dropout rate of 27%, primarily due to diarrhea[154].

Pharmacological interventions

Whereas non-pharmacological interventions are aimed at reducing the generation or improving the clearance of uraemic toxins, pharmacological interventions are

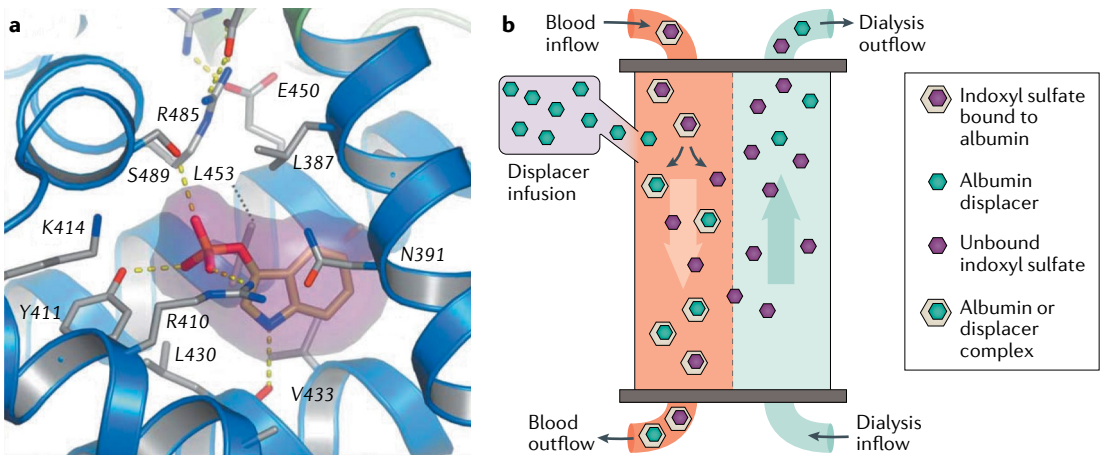

Fig. 5 | **Consequences of indoxyl sulfate binding to albumin. a** | Indoxyl sulfate interacts with human serum albumin at drug-binding site 2. Indoxyl sulfate is shown as a stick representation with a semi-transparent van der Waals surface (magenta). Selected side chains are shown as sticks color-coded by atom type; yellow dashed lines indicate hydrogen bonds. **b** | Indoxyl sulfate is a highly protein-bound uraemic toxin, and only ~10% exists in its free form. Indoxyl sulfate bound to albumin cannot be removed by dialysis; however, infusion of a ligand that can displace the uraemic toxin from the binding cavity of albumin is likely to increase the proportion of free indoxyl sulfate, enabling its removal in the dialysate outflow. Part **a** reprinted with permission from REF.[148], Elsevier.

Xenobiotic
A chemical substance found within an organism that is not naturally produced or expected to be present within the organism or in an ecological system.

generally aimed at reducing the downstream intracellular effects of uraemic toxins without altering their concentrations. The ideal pharmacological regimen is scalable, sustainable over a long period of time and can be targeted to a specific disease manifestation induced by a particular uraemic toxin or a group of uraemic toxins.

Klotho

Klotho is a membrane-bound protein that is highly expressed in kidney tubules but also exists as a secreted form[155]. Membrane-bound Klotho is a coreceptor for FGF23 and modulates FGF23 signal transduction. Soluble Klotho is found in the blood, urine, cerebrospinal fluid and is involved in processes such as aging, energy metabolism, Wnt signalling, anti-oxidation, ion transport and in the regulation of parathyroid hormone, the renin–angiotensin–aldosterone system and $1,25(OH)_2VD_3$ production[155]. Klotho has also demonstrated anti-senescent properties in several organ systems including aortic valve[156], regenerating muscle[157] and even adipose-derived stem cells[158]. Transgenic overexpression of Klotho expression in mouse models of CKD reduces vascular calcification, whereas Klotho haploinsufficiency has the opposite effect[159]. CKD is a state of Klotho deficiency[160] and PB uraemic toxins directly influence Klotho expression. Both indoxyl sulfate and *p*-cresyl sulfate hypermethylate the promoter of *Kl*, which results in downregulation of Klotho, exacerbation of renal fibrosis and deterioration of renal function in mice[161].

Administration of recombinant α-Klotho protein reduced kidney and cardiac fibrosis, attenuated cardiac remodelling and improved cardiac function in a mouse model of CKD[162]. In vitro and in vivo studies have shown that Klotho also protects against indoxyl sulfate-associated cardiac toxicity and left ventricular hypertrophy, with Klotho-deficient mice showing an exaggerated response to indoxyl sulfate[163]. In patients with CKD, lower serum Klotho levels were associated with arterial stiffness even after correcting for confounding factors[164]. By contrast, a 2.6-year follow-up study of patients with CKD stages 2–4 found that FGF23, but not Klotho itself, was associated with decompensated heart failure, although neither marker predicted development of atherosclerosis[165].

The link between Klotho and uraemic solutes remains poorly explored. Daily intraperitoneal injection of indoxyl sulfate for 8 weeks induced ventricular hypertrophy in mice — an effect that was more pronounced in Klotho-haplodeficient mice[163]. This ventricular hypertrophy was attenuated by administration of Klotho through inhibition of indoxyl sulfate-induced p38 and ROS signalling[163].

Ongoing clinical trials are aimed at assessing the value of serum Klotho as a predictor of cardiovascular calcification in patients with CKD[166]. It is conceivable that Klotho supplementation or use of a peptide-based approach may have beneficial effects in patients with CKD, including on the progression of kidney disease and cardiomyopathy[162]. However, additional studies are warranted to assess the beneficial effects of Klotho on uraemic toxin levels and CVD outcomes in patients with CKD.

Aryl hydrocarbon receptor inhibitors

AHR signalling is a well-known xenobiotic pathway associated with chemical carcinogenesis. AHR is a receptor that is ubiquitously expressed in the cytosol of cells, which upon ligand binding undergoes nuclear translocation to induce the transcription of genes such as those encoding specific members of the cytochrome P450 family of microsomal enzymes (FIG. 4). A wealth of studies in rodents have uncovered a role for AHR signalling in cardiovascular functions, including cardiac myocyte function[167,168], angiogenesis (that is, vessel development from the existing vascular bed) and neovascularization (that is, vessel development from a naive bed)[169,170], blood pressure regulation[171,172], atherosclerosis[78], myocardial ischaemia–reperfusion injury and post-ischaemic conditioning[173], and stroke[109].

Mounting evidence also implicates AHR signalling as an important mediator of uraemic toxicity by a set of uraemic toxins that are particularly cardio- and vasculotoxic. Uraemic toxins derived from tryptophan amino acids, including indoxyl sulfate, indoxyl acetate, kynurenine, kynurenic acid, are all AHR ligands[174,175]. Several mouse and human studies have implicated CKD as a global state of AHR activation with particular involvement of the myocardium and vasculature[175,176]. As described earlier, indoxyl sulfate and kynurenine bind to the ligand-binding domain of AHR, leading to increased levels of tissue factor in ECs and VSMCs, while simultaneously AHR binds tissue factor to prevent its degradation[74] (FIG. 4). This bimodal action increases levels of tissue factor, which promotes thrombosis, upon spontaneous rupture of plaque, thus augmenting the risk of adverse cardiovascular events. The central role of AHR in cardiovascular functions, as well as its activation by uraemic toxins, provides a strong mechanistic rationale for therapeutic targeting of AHR in CKD. A number of AHR inhibitors are already in pre-clinical development. We have assessed the effects of AHR inhibitors in mouse models of vascular injury-associated thrombosis in mice with adenine-induced CKD and in mouse models of uraemic toxicity induced by exposure to indoxyl sulfate or kynurenine[73,77]. Emulating features seen in patients with CKD, these mouse models exhibited increased levels of tissue factor in the aorta and heightened thrombosis at baseline as demonstrated by a shortened time to occlusion of carotid blood flow following ferric chloride-induced vascular injury as a model of occlusive thrombosis; administration of AHR inhibitors downregulated tissue factor expression and suppressed thrombosis.

Further mechanistic probing of the AHR–tissue factor–thrombosis axis showed that ligand-activated AHR regulates TF and thrombosis through the regulation of STUB1 (also known as CHIP)[73] — a U-box-containing E3 ligase that ubiquitinates several substrate proteins[177]. Genetic manipulation of *STUB1* in VSMCs showed that loss of STUB1 activity reduced tissue factor ubiquitination and increased thrombosis[73]. YL-109, an analogue of 2-(4-amino-3-methylphenyl)benzothiazole, has been assessed in preclinical studies owing to its proposed tumour-suppressing properties. It is a partial AHR inhibitor and upregulates *STUB1* transcription[178].

An increase in STUB1 protein levels induced degradation of tissue factor and suppressed thrombosis in a flow loop system[73]. Importantly, the activation of STUB1 by YL-109 did not augment the risk of bleeding[73]. These studies suggest support of the pharmacological rationale for suppressing the AHR-STUB1 axis to downregulate tissue factor and thrombosis in patients with CKD.

At least three AHR inhibitors, including CH223191 and CB7993113, have been assessed in models of CKD, illustrating a number of intriguing points. First, AHR inhibitors effectively extended the time to occlusion in CKD mice to that observed in non-CKD mice following ferric chloride-induced vascular injury[73]. Notably, inhibition of AHR did not increase the bleeding time of CKD mice as this manipulation did not alter physiological haemostatic mechanisms[73]. By contrast, the antithrombotic actions of heparin are accompanied by substantially prolonged bleeding time in CKD mice, because agents such as heparin or oral anticoagulants perturb the haemostatic machinery in blood as part of their action[73]. These findings in mice corroborate observations in patients with CKD. Estimates suggest that every 30-ml/min decrease in creatinine clearance is associated with a 50% increase in the risk of major and minor bleeding[179]. Thus, AHR seems to represent a safe and CKD-specific antithrombotic target in uraemic mice[73]. The finding that this approach can lower the thrombotic risk induced by the CKD milieu to a range seen in non-CKD models is encouraging. Second, the finding that AHR inhibitors antagonize the effects of indoxyl sulfate to downregulate TF in VSMCs[72] encourages the exploration of uraemic toxins as biomarkers to guide AHR inhibitor therapy in patients with CKD[72].

Adverse and off-target effects of AHR. Examination of potential adverse effects is important when considering any protein target, as is an understanding of the limitations of rodent models for studying AHR biology, as rodent and human AHR exhibit different ligand selectivity and gene regulation[180,181]. For example, mouse AHR demonstrated 10-fold higher binding affinity for typical AHR ligands such as 2,3,7,8 tetrachlorodibenzo-*p*-dioxin than human AHR, whereas human AHR had higher binding for indirubin[180]. In the same vein, genes identified as differentially expressed between mouse and human AHR are known to be involved in a number of biological pathways, including cell proliferation and the inflammatory response[181]. These studies underscore a need to exercise caution in extrapolating mouse data to humans for AHR studies. Global *Ahr*-knockout mice are viable and fertile[182] but show vascular defects (patent ductus venosus) in liver[183] and a 24% reduction in platelet counts, illustrating the importance of AHR expression in certain organ or cell types[184]. As mentioned earlier, members of the cytochrome P450 system of microsomal enzymes are direct transcriptional targets of AHR[185]. These enzymes catalyse the first step in the metabolism of lipophilic xenobiotics or drugs to generate more water-soluble intermediate compounds that can be readily excreted, suggesting that AHR inhibitors could induce undesirable drug–drug interactions. In addition, AHR regulates the expression of other metabolic enzymes, such as aldehyde dehydrogenases (specifically *ALDH1A1* and *ALDH3A1*) and specific drug transporters, such as multidrug resistance-associated protein 4 (encoded by *ABCC4*) and P-glycoprotein[186]. Available evidence suggests that uraemic toxins influence the expression of certain drug transporters, which can affect drug pharmacokinetics. For example, indoxyl sulfate upregulates the expression of P-glycoprotein in human hepatoma (HepG2) cells. The same study also demonstrated that of patients with CKD who underwent heart or kidney transplantation, those with higher serum levels of indoxyl sulfate required higher doses of ciclosporin (a P-glycoprotein substrate) to obtain the cyclosporin target blood concentration[187]. Thus, the CKD milieu may influence the pharmacokinetics of various medications through modulation of AHR-mediated transcriptional processes[186] and AHR inhibitors may normalize this adverse effect of uraemic toxins on drug clearance mechanisms. However, the effect of AHR inhibitors on the pharmacokinetics of drugs and other endogenous compounds[188] and the potential for adverse drug–drug interactions require careful consideration.

Limitations of AHR inhibitors. So far, these inhibitors have been developed using either cell-based AHR activity assays (for example, the Bayer compound BAY218)[189], or by in silico screening or homology modelling (CH223191 and CB7993113)[190]. In the absence of a crystal structure of human AHR, proof of their binding to the AHR is lacking. The absence of this information is notable given the increasing recognition that a number of drugs do not bind to their intended protein targets, but rather elicit biological activity through off-target effects[191]. The availability of this critical information will help to drive the development of highly selective AHR inhibitors and confirm their mechanism of action. Moreover, not all AHR inhibitors have been assessed in models of CKD.

AHR inhibitors can be classified as complete antagonists (such as CB7993113) or partial antagonists (such as YL109)[192]. On the basis of their ability to selectively regulate AHR signalling, AHR ligands are classified as either selective AHR modulators[193] (for example, 3′,4′-dimethoxy-α-naphthoflavone[194] and 6,2′,4′-trimethoxyflavone[195]) or non-selective AHR modulators (for example, CH223191 and CB7993113). Several AHR inhibitors currently in cancer trials[196,197] are complete AHR antagonists. Selective AHR modulators remain unexplored for therapeutic use in clinical studies. However, our current understanding of the integral role of AHR in cardiovascular biology, its activation by uraemic toxins, and its association with uraemic complications, suggests that this receptor is a tantalizing therapeutic target for CKD-associated CVD.

Kynureninase

The tryptophan metabolite kynureninase is associated with CVD in the general population[60,198], and has demonstrated pro-thrombotic properties in a mouse model of CKD[77]. Accumulation of kynureninase is evident in early stages of CKD and progresses with CKD progression, reaching levels of 4.8–6.5 μM (normal <2 μM).

Aza-analogue
Chemical compounds in which a carbon atom is replaced by a nitrogen atom.

As mentioned earlier, kynurenine is generated by the catabolism of tryptophan by indoleamine 2,3-dioxygenase 1 (IDO1) or tryptophan 2,3-dioxygenase 2 (TDO2), and is further degraded by kynureninase[40]. Therapeutic strategies to target kynurenine include suppression of its production or approaches to stimulating its degradation. IDO1 and kynurenine have been attributed immunosuppressive properties through the activation of AHR, leading to the production of immune-tolerant dendritic cells and regulatory T cells. Tumour cells produce excessive kynurenine to evade immune recognition and suppression of kynurenine production has therefore been considered as a clinical approach in the oncology field[199]. Efforts to promote kynurenine degradation through use of pegylated-kynureninase have also been made[200]. However, caution is warranted with this agent, as kynureninase may increase levels of metabolites such as anthranilic acid and quinolinic acid (FIG. 1b). Both of these metabolites are uraemic toxins that are associated with augmented thrombosis in patients[77], and contribute to other systemic toxic effects, such as neurocognitive diseases[201,202]. Kynureninase supplementation has not been examined in models of CKD. Careful long-term pre-clinical CKD models and clinical studies involving patients with CKD are warranted to examine the risk–benefit profile of targeting kynureninase in CKD.

Kinase inhibitors

Kinases are well-established mediators of almost all cellular functions and are validated targets, mostly in the field of cancer treatment. Tyrosine kinases (including both receptor and non-receptor kinases, such as Src kinases) and serine/threonine kinases (such as AKT) have been implicated in various kidney pathological conditions, such as liver fibrosis, glomerulonephritis and diabetic kidney disease[203,204], and are also considered to mediate several CVD pathological conditions, such as cardio-pulmonary fibrosis and ischaemia–reperfusion injury[205]. Despite these connections, little is known about the link between uraemic toxins and kinases. Indoxyl sulfate has been shown to induce the release of microparticles from human umbilical vein endothelial cells via upregulation of the p38–mitogen-activated protein kinase signalling pathway[206], and such microparticles have been implicated in promoting thrombosis[207]. However, key questions as to the effect of uraemic toxins on kinases and the relevance to CKD-associated CVD remain unanswered. A variety of kinase inhibitors are under preclinical development and/or are FDA approved[208], suggesting that these could be repurposed for therapeutic purposes for CKD pending the outcomes of appropriate studies.

TMAO inhibitors

TMAO is a metabolite of choline and L-carnitine, which are processed through specific enzymes in the gut microbiome (FIG. 2). In addition to dietary interventions and probiotics, investigators have assessed the ability of antibiotics to reduce the production of TMAO, and demonstrated beneficial effects on TMAO levels and aortic plaque development in mice fed with a high L-carnitine and/or choline diet[209]. However, this approach is likely to induce non-specific alterations in the gut microbiome. Meldonium is an aza-analogue of GBB — the quaternary amine bio-precursor of L-carnitine — and competes with L-carnitine and GBB for γ-butyrobetaine hydroxylase and carnitine/organic cation transporter type 2 (OCTN2), which synthesizes TMAO[210]. Treatment of rats with meldonium significantly decreased intestinal microbiota-dependent production of TMA and TMAO from L-carnitine but not from choline[211]. The administration of meldonium together with L-carnitine significantly increased GBB concentration in blood plasma and in isolated rat small intestine perfusates. Meldonium did not influence bacterial growth or bacterial uptake of L-carnitine but significantly decreased TMA production by *K. pneumoniae*. DMB is an inhibitor of choline lyase (FIG. 2). Mice fed a western diet with or without 1.0% DMB in drinking water for 8 weeks showed significantly reduced plasma TMAO levels and improved cardiac function compared with mice fed a western diet only[116]. DMB also prevented an increase in levels of proinflammatory cytokines and interstitial fibrosis in the hearts of these mice. However, DMB cannot completely eliminate TMAO synthesis. A 2018 study reported that enalapril lowered TMAO levels by increasing its excretion without affecting its production or the gut microbiome, suggesting that a combined approach to reducing TMAO production while promoting TMAO elimination might be more effective than either approach alone[212]. Although encouraging, studies to date have been pre-clinical and involved rodent models with normal kidney function; hence, the ability of these compounds to prevent CKD-associated CVD in experimental models and human patients through modulation of TMAO levels is yet to be established.

AST-120

The role of the gut flora in generating precursors for some uraemic toxins has generated interest in pharmacological agents that are enterally active. Of these, AST-120 is probably best known. AST-120 is an oral sorbent comprising porous spherical carbon particles that are capable of non-specifically adsorbing several low-molecular-weight molecules (100–10,000 kDa). Intestinal adsorption of intermediate metabolites of indoxyl sulfate, p-Cresol sulfate and other uraemic toxins by AST-120 reduces their availability to mediate toxic downstream effects[213]. On the basis of this rationale, pre-clinical studies have been performed to assess the effects of AST-120 on various uraemic manifestations, including atherosclerosis, cardiac dysfunction and bone disease, as discussed elsewhere[214].

AST-120 has been approved for the treatment of uraemic symptoms and to delay progression of disease in CKD patients in Japan since 1991 (REF.[215]). However, human studies have yielded conflicting results. Two multinational, randomized, double-blind, placebo-controlled trials that evaluated the effects of AST-120 on the progression of CKD in 2,035 adults with moderate to severe CKD[216] found no difference between the intervention and placebo groups in the time taken to reach the primary end point (a composite of dialysis initiation, kidney transplantation and doubling of the serum

creatinine level). Although clinical studies have primarily focused on the progression of CKD, a retrospective analysis of nearly 200 patients with non-dialysis-dependent CKD found significantly lower aortic calcification in those treated with AST-120 (REFS[217,218]). Despite these beneficial effects, AST-120 has not been approved for use in the USA or Europe owing to concerns related to its non-specific binding to several low-molecular-weight molecules in the gut. Nonetheless, these studies have paved the way for the development of targeted binders of the uraemic toxin precursors in the gut.

Conclusions and future directions

Epidemiological studies indisputably identify CKD as an independent risk factor for various CVDs, implicating the existence of CKD-specific risk mediators. These mediators include uraemic toxins, such as indoxyl sulfate and kynurenine, AHR activation, immune dysfunction and gut dysbiosis, and hence a multipronged approach is warranted to target these processes. An increasing number of mechanistic studies demonstrate the existence of a causal link between uraemic toxins and CKD-associated CVD, and as our understanding of the pathogenic contribution of uraemic toxins to CVD unfolds, a parallel, comprehensive approach is needed to translate this knowledge to the clinical realm.

As the processes underlying CVD development begin in the early stages of CKD and CVD risk increases as CKD progresses, it stands to reason that early targeting of uraemic toxins should be considered. Conventional haemodialysis cannot efficiently remove vasculotoxic PTUBs, which may underlie the poor outcomes of patients with advanced CKD[219].

The unique environment of CKD demands a unique approach. A number of nodes have been identified through which the pathogenicity of uraemic toxins can potentially be targeted; however, further research into the cardiovascular benefits of these targeted approaches is needed. A multimodal approach is also likely to be needed, with adjustments according to the different stages of CKD. For example, levels of uraemic toxins could be regulated through nutrition and microbiota manipulations in early stages of CKD, whereas more aggressive manoeuvres to improve the clearance of uraemic toxins could be applied in patients with kidney failure through the use of selective dialyzers for uraemic toxins[31,32,220]. The pharmacological targeting of cellular pathways affected by uraemic toxins could span all stages of CKD and could potentially be guided by biomarkers of CVD.

The successful translation of discoveries to the clinical realm will be contingent on overcoming bottlenecks such as the altered pharmacokinetics of patients with CKD, and will require careful evaluation of the cost–benefit ratio of protracted therapy, as well as consideration of primary end points for clinical studies. These and other challenges can be overcome by a coordinated effort by the nephrology community, biotechnology industry and regulatory agencies. Such an approach is needed to address the enormous CVD burden of patients with CKD.

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1. Hill, N. R. et al. Global prevalence of chronic kidney disease — a systematic review and meta-analysis. *PLoS ONE* **11**, e0158765 (2016).
2. Centers for Disease Control and Prevention. *Chronic Kidney Disease Surveillance System — United States*. *CDC* https://nccd.cdc.gov/ckd/default.aspx (2021).
3. Xu, J., Murphy, S. L., Kochanek, K. D. & Bastian, B. A. Deaths: final data for 2013. *Natl Vital Stat. Rep.* **64**, 1–119 (2016).
4. Luyckx, V. A., Tonelli, M. & Stanifer, J. W. The global burden of kidney disease and the sustainable development goals. *Bull. World Health Organ.* **96**, 414–422D (2018).
5. Centers for Disease Control and Prevention. Chronic kidney disease in the United States, 2019 (CDC, 2019).
6. Whitman, I. R., Feldman, H. I. & Deo, R. CKD and sudden cardiac death: epidemiology, mechanisms, and therapeutic approaches. *J. Am. Soc. Nephrol.* **23**, 1929–1939 (2012).
7. Charytan, D. M. Introduction: cardiovascular disease in chronic kidney disease. *Semin. Nephrol.* **38**, 541 (2018).
8. Weaver, D. J. & Mitsnefes, M. Cardiovascular disease in children and adolescents with chronic kidney disease. *Semin. Nephrol.* **38**, 559–569 (2018).
9. Temgoua, M. N., Danwang, C., Agbor, V. N. & Noubiap, J. J. Prevalence, incidence and associated mortality of cardiovascular disease in patients with chronic kidney disease in low- and middle-income countries: a protocol for a systematic review and meta-analysis. *BMJ Open* **7**, e016412 (2017).
10. Mathew, R. O. et al. Diagnosis and management of atherosclerotic cardiovascular disease in chronic kidney disease: a review. *Kidney Int.* **91**, 797–807 (2017).
11. Chronic Kidney Disease Prognosis Consortium. et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* **375**, 2073–2081 (2010).
12. van der Velde, M. et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int.* **79**, 1341–1352 (2011).
13. Gansevoort, R. T. et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet* **382**, 339–352 (2013).
14. Bansal, N. et al. Absolute rates of heart failure, coronary heart disease, and stroke in chronic kidney disease: an analysis of 3 community-based cohort studies. *JAMA Cardiol.* **2**, 314–318 (2017).
15. Fox, C. S. et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *Lancet* **380**, 1662–1673 (2012).
16. Tonelli, M. et al. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. *Lancet* **380**, 807–814 (2012).
17. Natsuaki, M. et al. Renal function and effect of statin therapy on cardiovascular outcomes in patients undergoing coronary revascularization (from the CREDO-Kyoto PCI/CABG Registry Cohort-2). *Am. J. Cardiol.* **110**, 1568–1577 (2012).
18. Wanner, C. et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N. Engl. J. Med.* **353**, 238–248 (2005).
19. Angiolillo, D. J. et al. Impact of chronic kidney disease on platelet function profiles in diabetes mellitus patients with coronary artery disease taking dual antiplatelet therapy. *J. Am. Coll. Cardiol.* **55**, 1139–1146 (2010).
20. Sorensen, V. R., Heaf, J., Wehberg, S. & Sorensen, S. S. Survival benefit in renal transplantation despite high comorbidity. *Transplantation* **100**, 2160–2167 (2016).
21. Rana, A. et al. Survival benefit of solid-organ transplant in the United States. *JAMA Surg.* **150**, 252–259 (2015).
22. Pesavento, T. E. Kidney transplantation in the context of renal replacement therapy. *Clin. J. Am. Soc. Nephrol.* **4**, 2035–2039 (2009).
23. Rao, P. S. et al. Renal transplantation in elderly patients older than 70 years of age: results from the scientific registry of transplant recipients. *Transplantation* **83**, 1069–1074 (2007).
24. Bottomley, M. J. & Harden, P. N. Update on the long-term complications of renal transplantation. *Br. Med. Bull.* **106**, 117–134 (2013).
25. Gross, M. L. & Ritz, E. Hypertrophy and fibrosis in the cardiomyopathy of uremia–beyond coronary heart disease. *Semin. Dial.* **21**, 308–318 (2008).
26. Price, A. M. et al. Changes in blood pressure and arterial hemodynamics following living kidney donation. *Clin. J. Am. Soc. Nephrol.* **15**, 1330–1339 (2020).
27. Ting, S. M. et al. Functional cardiovascular reserve predicts survival pre-kidney and post-kidney transplantation. *J. Am. Soc. Nephrol.* **25**, 187–195 (2014).
28. Poesen, R. et al. The influence of renal transplantation on retained microbial-human co-metabolites. *Nephrol. Dial. Transpl.* **31**, 1721–1729 (2016).
29. Liabeuf, S. et al. Levels of indoxyl sulfate in kidney transplant patients, and the relationship with hard outcomes. *Circ. J.* **80**, 722–730 (2016).
30. Liabeuf, S., Cheddani, L. & Massy, Z. A. Uremic toxins and clinical outcomes: the impact of kidney transplantation. *Toxins* **10**, 229 (2018).
31. Masereeuw, R. & Verhaar, M. C. Innovations in approaches to remove uraemic toxins. *Nat. Rev. Nephrol.* **16**, 552–553 (2020).
32. Geremia, I. & Stamatialis, D. Innovations in dialysis membranes for improved kidney replacement therapy. *Nat. Rev. Nephrol.* **16**, 550–551 (2020).
33. Vanholder, R. et al. Uremic toxicity: present state of the art. *Int. J. Artif. Organs* **24**, 695–725 (2001).
34. Duranton, F. et al. Normal and pathologic concentrations of uremic toxins. *J. Am. Soc. Nephrol.* **23**, 1258–1270 (2012).
35. Vanholder, R. C. & Glorieux, G. L. An overview of uremic toxicity. *Hemodial. Int.* **7**, 156–161 (2003).
36. Tang, W. H. et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* **368**, 1575–1584 (2013).
37. Mathew, S. et al. The mechanism of phosphorus as a cardiovascular risk factor in CKD. *J. Am. Soc. Nephrol.* **19**, 1092–1105 (2008).
38. Vogt, I., Haffner, D. & Leifheit-Nestler, M. FGF23 and phosphate-cardiovascular toxins in CKD. *Toxins* **11**, 647 (2019).

39. Evenepoel, P., Poesen, R. & Meijers, B. The gut-kidney axis. *Pediatr. Nephrol.* **32**, 2005–2014 (2017).
40. Roager, H. M. & Licht, T. R. Microbial tryptophan catabolites in health and disease. *Nat. Commun.* **9**, 3294 (2018).
41. Kolodziej, L. R., Paleolog, E. M. & Williams, R. O. Kynurenine metabolism in health and disease. *Amino Acids* **41**, 1173–1183 (2011).
42. Saito, K., Crowley, J. S., Markey, S. P. & Heyes, M. P. A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. *J. Biol. Chem.* **268**, 15496–15503 (1993).
43. Chen, Y. & Guillemin, G. J. Kynurenine pathway metabolites in humans: disease and healthy states. *Int. J. Tryptophan Res.* **2**, 1–19 (2009).
44. Deguchi, T., Kouno, Y., Terasaki, T., Takadate, A. & Otagiri, M. Differential contributions of rOat1 (Slc22a6) and rOat3 (Slc22a8) to the in vivo renal uptake of uremic toxins in rats. *Pharm. Res.* **22**, 619–627 (2005).
45. Uwai, Y., Honjo, H. & Iwamoto, K. Interaction and transport of kynurenic acid via human organic anion transporters hOAT1 and hOAT3. *Pharmacol. Res.* **65**, 254–260 (2012).
46. Saito, K. et al. Mechanism of increases in L-kynurenine and quinolinic acid in renal insufficiency. *Am. J. Physiol. Renal Physiol.* **279**, F565–F572 (2000).
47. Tilg, H. A gut feeling about thrombosis. *N. Engl. J. Med.* **374**, 2494–2496 (2016).
48. Pelletier, C. C. et al. Elevation of trimethylamine-N-oxide in chronic kidney disease: contribution of decreased glomerular filtration rate. *Toxins* **11**, 635 (2019).
49. Block, G. A. et al. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J. Am. Soc. Nephrol.* **15**, 2208–2218 (2004).
50. Cheung, A. K. et al. Serum beta-2 microglobulin levels predict mortality in dialysis patients: results of the HEMO study. *J. Am. Soc. Nephrol.* **17**, 546–555 (2006).
51. Liabeuf, S. et al. Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. *Nephrol. Dial. Transpl.* **25**, 1183–1191 (2010).
52. Wu, I. W. et al. Serum free p-cresyl sulfate levels predict cardiovascular and all-cause mortality in elderly hemodialysis patients — a prospective cohort study. *Nephrol. Dial. Transpl.* **27**, 1169–1175 (2012).
53. Stubbs, J. R. et al. Serum trimethylamine-N-oxide is elevated in ckd and correlates with coronary atherosclerosis burden. *J. Am. Soc. Nephrol.* **27**, 305–313 (2016).
54. Wu, C. C., Hung, S. C., Kuo, K. L. & Tarng, D. C. Impact of indoxyl sulfate on progenitor cell-related neovascularization of peripheral arterial disease and post-angioplasty thrombosis of dialysis vascular access. *Toxins* **9**, 25 (2017).
55. Tomlinson, J. A. P. & Wheeler, D. C. The role of trimethylamine N-oxide as a mediator of cardiovascular complications in chronic kidney disease. *Kidney Int.* **92**, 809–815 (2017).
56. Senthong, V. et al. Intestinal microbiota-generated metabolite trimethylamine-N-oxide and 5-year mortality risk in stable coronary artery disease: the contributory role of intestinal microbiota in a COURAGE-like patient cohort. *J. Am. Heart Assoc.* **5**, e002816 (2016).
57. Lin, C. J. et al. Indoxyl sulfate predicts cardiovascular disease and renal function deterioration in advanced chronic kidney disease. *Arch. Med. Res.* **43**, 451–456 (2012).
58. Fan, P. C. et al. Serum indoxyl sulfate predicts adverse cardiovascular events in patients with chronic kidney disease. *J. Formos. Med. Assoc.* **118**, 1099–1106 (2019).
59. Meijers, B. K. et al. Free p-cresol is associated with cardiovascular disease in hemodialysis patients. *Kidney Int.* **73**, 1174–1180 (2008).
60. Pedersen, E. R. et al. Associations of plasma kynurenines with risk of acute myocardial infarction in patients with stable angina pectoris. *Arterioscler. Thromb. Vasc. Biol.* **35**, 455–462 (2015).
61. Dou, L. et al. The cardiovascular effect of the uremic solute indole-3 acetic acid. *J. Am. Soc. Nephrol.* **26**, 876–887 (2015).
62. Foley, R. N. et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney Int.* **47**, 186–192 (1995).
63. Yang, K. et al. Indoxyl sulfate induces oxidative stress and hypertrophy in cardiomyocytes by inhibiting the AMPK/UCP2 signaling pathway. *Toxicol. Lett.* **234**, 110–119 (2015).
64. Yisireyili, M. et al. Indoxyl sulfate promotes cardiac fibrosis with enhanced oxidative stress in hypertensive rats. *Life Sci.* **92**, 1180–1185 (2013).
65. Han, H. et al. p-Cresyl sulfate aggravates cardiac dysfunction associated with chronic kidney disease by enhancing apoptosis of cardiomyocytes. *J. Am. Heart Assoc.* **4**, e001852 (2015).
66. Rysz, J., Franczyk, B., Lawinski, J. & Gluba-Brzozka, A. Oxidative stress in ESRD patients on dialysis and the risk of cardiovascular diseases. *Antioxidants* **9**, 1079 (2020).
67. Cybulsky, A. V. Endoplasmic reticulum stress in proteinuric kidney disease. *Kidney Int.* **77**, 187–193 (2010).
68. Dou, L. et al. The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney Int.* **65**, 442–451 (2004).
69. Meijers, B. K. et al. The uremic retention solute p-cresyl sulfate and markers of endothelial damage. *Am. J. Kidney Dis.* **54**, 891–901 (2009).
70. Lin, C. J. et al. Indoxyl sulfate impairs endothelial progenitor cells and might contribute to vascular dysfunction in patients with chronic kidney disease. *Kidney Blood Press. Res.* **41**, 1025–1036 (2016).
71. De Deyn, P. P., Vanholder, R. & D'Hooge, R. Nitric oxide in uremia: effects of several potentially toxic guanidino compounds. *Kidney Int. Suppl.* **84**, S25–S28 (2003).
72. Shivanna, S. et al. The aryl hydrocarbon receptor is a critical regulator of tissue factor stability and an antithrombotic target in uremia. *J. Am. Soc. Nephrol.* **27**, 189–201 (2016).
73. Shashar, M. et al. Targeting STUB1-tissue factor axis normalizes hyperthrombotic uremic phenotype without increasing bleeding risk. *Sci. Transl Med.* **9**, 1–11 (2017).
74. Gondouin, B. et al. Indolic uremic solutes increase tissue factor production in endothelial cells by the aryl hydrocarbon receptor pathway. *Kidney Int.* **84**, 733–744 (2013).
75. Karbowska, M. et al. Indoxyl sulfate promotes arterial thrombosis in rat model via increased levels of complex TF/VII, PAI-1, platelet activation as well as decreased contents of SIRT1 and SIRT3. *Front. Physiol.* **9**, 1623 (2018).
76. Chitalia, V. C. et al. Uremic serum and solutes increase post-vascular interventional thrombotic risk through altered stability of smooth muscle cell tissue factor. *Circulation* **127**, 365–376 (2013).
77. Kolachalama, V. B. et al. Uremic solute-aryl hydrocarbon receptor-tissue factor axis associates with thrombosis after vascular injury in humans. *J. Am. Soc. Nephrol.* **29**, 1063–1072 (2018).
78. Wu, D. et al. Activation of aryl hydrocarbon receptor induces vascular inflammation and promotes atherosclerosis in apolipoprotein $E^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* **31**, 1260–1267 (2011).
79. Henaut, L., Mary, A., Chillon, J. M., Kamel, S. & Massy, Z. A. The impact of uremic toxins on vascular smooth muscle cell function. *Toxins* **10**, 218 (2018).
80. Yang, K. et al. Indoxyl sulfate induces platelet hyperactivity and contributes to chronic kidney disease-associated thrombosis in mice. *Blood* **129**, 2667–2679 (2017).
81. Karbowska, M. et al. The uremic toxin indoxyl sulfate accelerates thrombotic response after vascular injury in animal models. *Toxins* **9**, 229 (2017).
82. Wang, X. & Shapiro, J. I. Evolving concepts in the pathogenesis of uraemic cardiomyopathy. *Nat. Rev. Nephrol.* **15**, 159–175 (2019).
83. Eloot, S. et al. Protein-bound uremic toxin profiling as a tool to optimize hemodialysis. *PLoS ONE* **11**, e0147159 (2016).
84. Leong, S. C. & Sirich, T. L. Indoxyl sulfate-review of toxicity and therapeutic strategies. *Toxins* **8**, 358 (2016).
85. Gryp, T., Vanholder, R., Vaneechoutte, M. & Glorieux, G. p-Cresyl Sulfate. *Toxins* **9**, 52 (2017).
86. Barreto, F. C. et al. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clin. J. Am. Soc. Nephrol.* **4**, 1551–1558 (2009).
87. Debnath, S. et al. Tryptophan metabolism in patients with chronic kidney disease secondary to type 2 diabetes: relationship to inflammatory markers. *Int. J. Tryptophan Res.* **10**, 1178646917694600 (2017).
88. Shivanna, S. et al. The aryl hydrocarbon receptor is a critical regulator of tissue factor stability and an antithrombotic target in uremia. *J. Am. Soc. Nephrol.* **27**, 187–201 (2016).
89. Vanholder, R. & Glorieux, G. Introduction: uremic toxicity — state of the art 2014. *Semin. Nephrol.* **34**, 85–86 (2014).
90. Vanholder, R., Schepers, E., Pletinck, A., Nagler, E. V. & Glorieux, G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. *J. Am. Soc. Nephrol.* **25**, 1897–1907 (2014).
91. Addi, T., Dou, L. & Burtey, S. Tryptophan-derived uremic toxins and thrombosis in chronic kidney disease. *Toxins* **10**, 412 (2018).
92. Konopelski, P. & Ufnal, M. Indoles — gut bacteria metabolites of tryptophan with pharmacotherapeutic potential. *Curr. Drug Metab.* **19**, 883–890 (2018).
93. Mitch, W. E. & Remuzzi, G. Diets for patients with chronic kidney disease, should we reconsider? *BMC Nephrol.* **17**, 80 (2016).
94. Carrero, J. J. et al. Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat. Rev. Nephrol.* **16**, 525–542 (2020).
95. Brunori, G. et al. Efficacy and safety of a very-low-protein diet when postponing dialysis in the elderly: a prospective randomized multicenter controlled study. *Am. J. Kidney Dis.* **49**, 569–580 (2007).
96. Fouque, D., Wang, P., Laville, M. & Boissel, J. P. Low protein diets delay end-stage renal disease in non diabetic adults with chronic renal failure. *Cochrane Database Syst. Rev.* **2**,CD001892 (2000).
97. Poesen, R. et al. The influence of dietary protein intake on mammalian tryptophan and phenolic metabolites. *PLoS ONE* **10**, e0140820 (2015).
98. Rhee, C. M., Ahmadi, S. F., Kovesdy, C. P. & Kalantar-Zadeh, K. Low-protein diet for conservative management of chronic kidney disease: a systematic review and meta-analysis of controlled trials. *J. Cachexia Sarcopenia Muscle* **9**, 235–245 (2018).
99. Menon, V. et al. Effect of a very low-protein diet on outcomes: long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. *Am. J. Kidney Dis.* **53**, 208–217 (2009).
100. Levey, A. S. et al. Effect of dietary protein restriction on the progression of kidney disease: long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. *Am. J. Kidney Dis.* **48**, 879–888 (2006).
101. Li, A., Lee, H. Y. & Lin, Y. C. The effect of ketoanalogues on chronic kidney disease deterioration: a meta-analysis. *Nutrients* **11**, 957 (2019).
102. Kontessis, P. et al. Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney Int.* **38**, 136–144 (1990).
103. Azadbakht, L., Atabak, S. & Esmaillzadeh, A. Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy: a longitudinal randomized clinical trial. *Diabetes Care* **31**, 648–654 (2008).
104. Barcenilla, A. et al. Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl. Env. Microbiol.* **66**, 1654–1661 (2000).
105. Nicholson, J. K. et al. Host-gut microbiota metabolic interactions. *Science* **336**, 1262–1267 (2012).
106. Pignanelli, M. et al. Mediterranean diet score: associations with metabolic products of the intestinal microbiome, carotid plaque burden, and renal function. *Nutrients* **10**, 779 (2018).
107. Boutagy, N. E. et al. Short-term high-fat diet increases postprandial trimethylamine-N-oxide in humans. *Nutr. Res.* **35**, 858–864 (2015).
108. Li, T., Chen, Y., Gua, C. & Li, X. Elevated circulating trimethylamine N-oxide levels contribute to endothelial dysfunction in aged rats through vascular inflammation and oxidative stress. *Front. Physiol.* **8**, 350 (2017).
109. Koeth, R. A. et al. gamma-Butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. *Cell Metab.* **20**, 799–812 (2014).
110. Janeiro, M. H., Ramirez, M. J., Milagro, F. I., Martinez, J. A. & Solas, M. Implication of trimethylamine N-oxide (TMAO) in disease: potential biomarker or new therapeutic target. *Nutrients* **10**, 1398 (2018).
111. Estruch, R. et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N. Engl. J. Med.* **378**, e34 (2018).
112. Wu, Wei-Kai, Chi-Tang Hoc, S. P., Kuod, Ching-Hua, Wub, Ming-Shiang & Shee, Lee-Yan Dietary allicin reduces transformation of L-carnitine to TMAO through impact on gut microbiota. *J. Funct. Foods* **15**, 408–417 (2015).

113. Chan, J. Y., Yuen, A. C., Chan, R. Y. & Chan, S. W. A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytother. Res.* **27**, 637–646 (2013).
114. Shi, X. et al. Allicin improves metabolism in high-fat diet-induced obese mice by modulating the gut microbiota. *Nutrients* **11**, 2909 (2019).
115. Wang, Z. et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* **163**, 1585–1595 (2015).
116. Chen, K., Zheng, X., Feng, M., Li, D. & Zhang, H. Gut microbiota-dependent metabolite trimethylamine N-oxide contributes to cardiac dysfunction in western diet-induced obese mice. *Front. Physiol.* **8**, 139 (2017).
117. Koeth, R. A. et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J. Clin. Invest.* **129**, 373–387 (2019).
118. Conlon, M. A. & Bird, A. R. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* **7**, 17–44 (2014).
119. Santisteban, M. M. et al. Hypertension-linked pathophysiological alterations in the gut. *Circ. Res.* **120**, 312–323 (2017).
120. Jie, Z. et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat. Commun.* **8**, 845 (2017).
121. Ianiro, G., Tilg, H. & Gasbarrini, A. Antibiotics as deep modulators of gut microbiota: between good and evil. *Gut* **65**, 1906–1915 (2016).
122. Andersen, K. et al. Intestinal dysbiosis, barrier dysfunction, and bacterial translocation account for CKD-related systemic inflammation. *J. Am. Soc. Nephrol.* **28**, 76–83 (2017).
123. Wong, J. et al. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am. J. Nephrol.* **39**, 230–237 (2014).
124. Goncalves, S. et al. Associations between renal function, volume status and endotoxaemia in chronic kidney disease patients. *Nephrol. Dial. Transpl.* **21**, 2788–2794 (2006).
125. Raij, L., Shapiro, F. L. & Michael, A. F. Endotoxemia in febrile reactions during hemodialysis. *Kidney Int.* **4**, 57–60 (1973).
126. Meijers, B., Evenepoel, P. & Anders, H. J. Intestinal microbiome and fitness in kidney disease. *Nat. Rev. Nephrol.* **15**, 531–545 (2019).
127. Mafra, D. et al. Food as medicine: targeting the uraemic phenotype in chronic kidney disease. *Nature Rev. Nephrol.* **17**, 153–171 (2021).
128. Salmean, Y. A., Segal, M. S., Palii, S. P. & Dahl, W. J. Fiber supplementation lowers plasma p-cresol in chronic kidney disease patients. *J. Ren. Nutr.* **25**, 316–320 (2015).
129. Evenepoel, P., Bammens, B., Verbeke, K. & Vanrenterghem, Y. Acarbose treatment lowers generation and serum concentrations of the protein-bound solute p-cresol: a pilot study. *Kidney Int.* **70**, 192–198 (2006).
130. Mishima, E. et al. Canagliflozin reduces plasma uremic toxins and alters the intestinal microbiota composition in a chronic kidney disease mouse model. *Am. J. Physiol. Renal Physiol.* **315**, F824–F833 (2018).
131. Marques, F. Z. et al. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation* **135**, 964–977 (2017).
132. Wilck, N. et al. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature* **551**, 585–589 (2017).
133. Borges, N. A. et al. Probiotic supplementation in chronic kidney disease: a double-blind, randomized, placebo-controlled trial. *J. Ren. Nutr.* **28**, 28–36 (2018).
134. Rossi, M. et al. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): a randomized trial. *Clin. J. Am. Soc. Nephrol.* **11**, 223–231 (2016).
135. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02302287 (2016).
136. Din, A. U. et al. Amelioration of TMAO through probiotics and its potential role in atherosclerosis. *Appl. Microbiol. Biotechnol.* **103**, 9217–9228 (2019).
137. Boutagy, N. E. et al. Probiotic supplementation and trimethylamine-N-oxide production following a high-fat diet. *Obesity* **23**, 2357–2363 (2015).
138. Yacoub, R. & Wyatt, C. M. Manipulating the gut microbiome to decrease uremic toxins. *Kidney Int.* **91**, 521–523 (2017).
139. Devlin, A. S. et al. Modulation of a circulating uremic solute via rational genetic manipulation of the gut microbiota. *Cell Host Microbe* **20**, 709–715 (2016).
140. Roberts, A. B. et al. Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat. Med.* **24**, 1407–1417 (2018).
141. Colosimo, D. A. et al. Mapping interactions of microbial metabolites with human G-protein-coupled receptors. *Cell Host Microbe* **26**, 273–282.e7 (2019).
142. Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* **449**, 811–818 (2007).
143. Gryp, T. et al. Gut microbiota generation of protein-bound uremic toxins and related metabolites is not altered at different stages of chronic kidney disease. *Kidney Int.* **97**, 1230–1242 (2020).
144. Ascenzi, P. & Fasano, M. Allostery in a monomeric protein: the case of human serum albumin. *Biophys. Chem.* **148**, 16–22 (2010).
145. Sarnatskaya, V. V. et al. Effect of protein-bound uraemic toxins on the thermodynamic characteristics of human albumin. *Biochem. Pharmacol.* **63**, 1287–1296 (2002).
146. Meijers, B. K., Bammens, B., Verbeke, K. & Evenepoel, P. A review of albumin binding in CKD. *Am. J. Kidney Dis.* **51**, 839–850 (2008).
147. Zaidi, N., Ajmal, M. R., Rabbani, G., Ahmad, E. & Khan, R. H. A comprehensive insight into binding of hippuric acid to human serum albumin: a study to uncover its impaired elimination through hemodialysis. *PLoS ONE* **8**, e71422 (2013).
148. Ghuman, J. et al. Structural basis of the drug-binding specificity of human serum albumin. *J. Mol. Biol.* **353**, 38–52 (2005).
149. Meyer, T. W. The removal of protein-bound solutes by dialysis. *J. Ren. Nutr.* **22**, 203–206 (2012).
150. Krieter, D. H. et al. Protein-bound uraemic toxin removal in haemodialysis and post-dilution haemodiafiltration. *Nephrol. Dial. Transpl.* **25**, 212–218 (2010).
151. Niwa, T. Removal of protein-bound uraemic toxins by haemodialysis. *Blood Purif.* **35**, 20–25 (2013).
152. Meijers, B. K. et al. Major coagulation disturbances during fractionated plasma separation and adsorption. *Am. J. Transpl.* **7**, 2195–2199 (2007).
153. Tao, X. et al. Improved dialytic removal of protein-bound uraemic toxins with use of albumin binding competitors: an in vitro human whole blood study. *Sci. Rep.* **6**, 23389 (2016).
154. Sakaguchi, Y. et al. A randomized trial of magnesium oxide and oral carbon adsorbent for coronary artery calcification in predialysis CKD. *J. Am. Soc. Nephrol.* **30**, 1073–1085 (2019).
155. Kuro, O. M. The Klotho proteins in health and disease. *Nat. Rev. Nephrol.* **15**, 27–44 (2019).
156. Chen, J., Fan, J., Wang, S. & Sun, Z. Secreted klotho attenuates inflammation-associated aortic valve fibrosis in senescence-accelerated mice P1. *Hypertension* **71**, 877–885 (2018).
157. Sahu, A. et al. Age-related declines in alpha-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration. *Nat. Commun.* **9**, 4859 (2018).
158. Fan, J. & Sun, Z. The antiaging gene Klotho regulates proliferation and differentiation of adipose-derived stem cells. *Stem Cell* **34**, 1615–1625 (2016).
159. Hu, M. C. et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J. Am. Soc. Nephrol.* **22**, 124–136 (2011).
160. Rotondi, S. et al. Soluble alpha-klotho serum levels in chronic kidney disease. *Int. J. Endocrinol.* **2015**, 872193 (2015).
161. Sun, C. Y., Chang, S. C. & Wu, M. S. Suppression of Klotho expression by protein-bound uremic toxins is associated with increased DNA methyltransferase expression and DNA hypermethylation. *Kidney Int.* **81**, 640–650 (2012).
162. Hu, M. C. et al. Recombinant alpha-Klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy. *Kidney Int.* **91**, 1104–1114 (2017).
163. Yang, K. et al. Klotho protects against indoxyl sulphate-induced myocardial hypertrophy. *J. Am. Soc. Nephrol.* **26**, 2434–2446 (2015).
164. Kitagawa, M. et al. A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. *PLoS ONE* **8**, e56695 (2013).
165. Seiler, S. et al. Associations of FGF-23 and sKlotho with cardiovascular outcomes among patients with CKD stages 2–4. *Clin. J. Am. Soc. Nephrol.* **9**, 1049–1058 (2014).
166. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03996746 (2020).
167. Lund, A. K., Goens, M. B., Nunez, B. A. & Walker, M. K. Characterizing the role of endothelin-1 in the progression of cardiac hypertrophy in aryl hydrocarbon receptor (AhR) null mice. *Toxicol. Appl. Pharmacol.* **212**, 127–135 (2006).
168. Sauzeau, V. et al. Transcriptional factor aryl hydrocarbon receptor (Ahr) controls cardiovascular and respiratory functions by regulating the expression of the Vav3 proto-oncogene. *J. Biol. Chem.* **286**, 2896–2909 (2011).
169. Juan, S. H., Lee, J. L., Ho, P. Y., Lee, Y. H. & Lee, W. S. Antiproliferative and antiangiogenic effects of 3-methylcholanthrene, an aryl-hydrocarbon receptor agonist, in human umbilical vascular endothelial cells. *Eur. J. Pharmacol.* **530**, 1–8 (2006).
170. Ichihara, S. et al. A role for the aryl hydrocarbon receptor in regulation of ischemia-induced angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1297–1304 (2007).
171. Vasquez, A. et al. A role for the aryl hydrocarbon receptor in cardiac physiology and function as demonstrated by AhR knockout mice. *Cardiovasc. Toxicol.* **3**, 153–163 (2003).
172. Agbor, L. N., Elased, K. M. & Walker, M. K. Endothelial cell-specific aryl hydrocarbon receptor knockout mice exhibit hypotension mediated, in part, by an attenuated angiotensin II responsiveness. *Biochem. Pharmacol.* **82**, 514–523 (2011).
173. Xue, Y. et al. Baicalin inhibits inflammation and attenuates myocardial ischaemic injury by aryl hydrocarbon receptor. *J. Pharm. Pharmacol.* **67**, 1756–1764 (2015).
174. Schroeder, J. C. et al. The uremic toxin 3-indoxyl sulfate is a potent endogenous agonist for the human aryl hydrocarbon receptor. *Biochemistry* **49**, 393–400 (2010).
175. Dou, L. et al. Aryl hydrocarbon receptor is activated in patients and mice with chronic kidney disease. *Kidney Int.* **93**, 986–999 (2018).
176. Walker, J. A. et al. Temporal and tissue-specific activation of aryl hydrocarbon receptor in discrete mouse models of kidney disease. *Kidney Int.* **97**, 538–550 (2020).
177. McDonough, H. & Patterson, C. CHIP: a link between the chaperone and proteasome systems. *Cell Stress Chaperones* **8**, 303–308 (2003).
178. Hiyoshi, H. et al. 2-(4-Hydroxy-3-methoxyphenyl)-benzothiazole suppresses tumor progression and metastatic potential of breast cancer cells by inducing ubiquitin ligase CHIP. *Sci. Rep.* **4**, 7095 (2014).
179. Fox, K. A. et al. The impact of renal dysfunction on outcomes in the ExTRACT-TIMI 25 trial. *J. Am. Coll. Cardiol.* **49**, 2249–2255 (2007).
180. Flaveny, C. A. & Perdew, G. H. Transgenic humanized AHR mouse reveals differences between human and mouse AHR ligand selectivity. *Mol. Cell Pharmacol.* **1**, 119–123 (2009).
181. Flaveny, C. A., Murray, I. A. & Perdew, G. H. Differential gene regulation by the human and mouse aryl hydrocarbon receptor. *Toxicol. Sci.* **114**, 217–225 (2010).
182. Chang, X. et al. Ligand-independent regulation of transforming growth factor beta1 expression and cell cycle progression by the aryl hydrocarbon receptor. *Mol. Cell Biol.* **27**, 6127–6139 (2007).
183. Bunger, M. K. et al. Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor. *Toxicol. Sci.* **106**, 83–92 (2008).
184. Lindsey, S., Jiang, J., Woulfe, D. & Papoutsakis, E. T. Platelets from mice lacking the aryl hydrocarbon receptor exhibit defective collagen-dependent signaling. *J. Thromb. Haemost.* **12**, 383–394 (2014).
185. Fujii-Kuriyama, Y. & Mimura, J. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem. Biophys. Res. Commun.* **338**, 311–317 (2005).
186. Santana Machado, T., Cerini, C. & Burtey, S. Emerging roles of aryl hydrocarbon receptors in the altered clearance of drugs during chronic kidney disease. *Toxins* **11**, 209 (2019).
187. Santana Machado, T. et al. Indoxyl sulfate upregulates liver P-glycoprotein expression and activity through aryl hydrocarbon receptor signaling. *J. Am. Soc. Nephrol.* **29**, 906–918 (2018).

188. Hankinson, O. The role of AHR-inducible cytochrome P450s in metabolism of polyunsaturated fatty acids. *Drug Metab. Rev.* **48**, 342–350 (2016).
189. Schmees, N. et al. Identification of BAY-218, a potent and selective small-molecule AhR inhibitor, as a new modality to counteract tumor immunosuppression [abstract 4454]. *Cancer Res.* **79** (Suppl. 13), 4454 (2019).
190. Parks, A. J. et al. In silico identification of an aryl hydrocarbon receptor antagonist with biological activity in vitro and in vivo. *Mol. Pharmacol.* **86**, 593–608 (2014).
191. Lin, A. et al. Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. *Sci. Transl Med.* **11**, eaaw8412 (2019).
192. Smith, K. J. et al. Identification of a high-affinity ligand that exhibits complete aryl hydrocarbon receptor antagonism. *J. Pharmacol. Exp. Ther.* **338**, 318–327 (2011).
193. Murray, I. A. et al. Evidence for ligand-mediated selective modulation of aryl hydrocarbon receptor activity. *Mol. Pharmacol.* **77**, 247–254 (2010).
194. Murray, I. A. et al. Suppression of cytokine-mediated complement factor gene expression through selective activation of the Ah receptor with 3′,4′-dimethoxy-alpha-naphthoflavone. *Mol. Pharmacol.* **79**, 508–519 (2011).
195. Murray, I. A. et al. Antagonism of aryl hydrocarbon receptor signaling by 6,2′,4′-trimethoxyflavone. *J. Pharmacol. Exp. Ther.* **332**, 135–144 (2010).
196. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04069026 (2021).
197. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04200963 (2020).
198. Zuo, H. et al. Plasma biomarkers of inflammation, the kynurenine pathway, and risks of all-cause, cancer, and cardiovascular disease mortality: the hordaland health study. *Am. J. Epidemiol.* **183**, 249–258 (2016).
199. Prendergast, G. C., Malachowski, W. P., DuHadaway, J. B. & Muller, A. J. Discovery of IDO1 inhibitors: from bench to bedside. *Cancer Res.* **77**, 6795–6811 (2017).
200. Triplett, T. A. et al. Reversal of indoleamine 2,3-dioxygenase-mediated cancer immune suppression by systemic kynurenine depletion with a therapeutic enzyme. *Nat. Biotechnol.* **36**, 758–764 (2018).
201. Lugo-Huitron, R. et al. Quinolinic acid: an endogenous neurotoxin with multiple targets. *Oxid. Med. Cell. Longev.* **2013**, 104024 (2013).
202. Bugnicourt, J. M., Godefroy, O., Chillon, J. M., Choukroun, G. & Massy, Z. A. Cognitive disorders and dementia in CKD: the neglected kidney-brain axis. *J. Am. Soc. Nephrol.* **24**, 353–363 (2013).
203. Wang, J. & Zhuang, S. Src family kinases in chronic kidney disease. *Am. J. Physiol. Renal Physiol* **313**, F721–F728 (2017).
204. Lan, A. & Du, J. Potential role of Akt signaling in chronic kidney disease. *Nephrol. Dial. Transpl.* **30**, 385–394 (2015).
205. Shahin, R., Shaheen, O., El-Dahiyat, F., Habash, M. & Saffour, S. Research advances in kinase enzymes and inhibitors for cardiovascular disease treatment. *Future Sci. OA* **3**, FSO204 (2017).
206. Ryu, J. H. & Kim, S. J. Clopidogrel effectively suppresses endothelial microparticle generation induced by indoxyl sulfate via inhibition of the p38 mitogen-activated protein kinase pathway. *Blood Purif.* **32**, 186–194 (2011).
207. Owens, A. P. 3rd. & Mackman, N. Microparticles in hemostasis and thrombosis. *Circ. Res.* **108**, 1284–1297 (2011).
208. Wu, P., Nielsen, T. E. & Clausen, M. H. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol. Sci.* **36**, 422–439 (2015).
209. Koeth, R. A. et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **19**, 576–585 (2013).
210. Kuka, J. et al. The cardioprotective effect of mildronate is diminished after co-treatment with L-carnitine. *J. Cardiovasc. Pharmacol. Ther.* **17**, 215–222 (2012).
211. Kuka, J. et al. Suppression of intestinal microbiota-dependent production of pro-atherogenic trimethylamine N-oxide by shifting L-carnitine microbial degradation. *Life Sci.* **117**, 84–92 (2014).
212. Konop, M. et al. Enalapril decreases rat plasma concentration of TMAO, a gut bacteria-derived cardiovascular marker. *Biomarkers* **23**, 380–385 (2018).
213. Sato, E. et al. Impact of the oral adsorbent AST-120 on organ-specific accumulation of uremic toxins: LC-MS/MS and MS imaging techniques. *Toxins* **10**, 19 (2017).
214. Liu, W. C., Tomino, Y. & Lu, K. C. Impacts of indoxyl sulfate and p-cresol sulfate on chronic kidney disease and mitigating effects of AST-120. *Toxins* **10**, 367 (2018).
215. Asai, M., Kumakura, S. & Kikuchi, M. Review of the efficacy of AST-120 (KREMEZIN®) on renal function in chronic kidney disease patients. *Ren. Fail.* **41**, 47–56 (2019).
216. Yamaguchi, J., Tanaka, T. & Inagi, R. Effect of AST-120 in chronic kidney disease treatment: still a controversy? *Nephron* **135**, 201–206 (2017).
217. Schulman, G. et al. Randomized placebo-controlled EPPIC trials of AST-120 in CKD. *J. Am. Soc. Nephrol.* **26**, 1732–1746 (2015).
218. Goto, S. et al. Association between AST-120 and abdominal aortic calcification in predialysis patients with chronic kidney disease. *Clin. Exp. Nephrol.* **17**, 365–371 (2013).
219. Eknoyan, G. et al. Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N. Engl. J. Med.* **347**, 2010–2019 (2002).
220. Wieringa, F. P. & Kooman, J. P. Smart sensors for real-time monitoring of patients on dialysis. *Nat. Rev. Nephrol.* **16**, 554–555 (2020).

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