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Review article

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Gut microbiome studies in CKD: opportunities, pitfalls and therapeutic potential

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Abstract

Interest in gut microbiome dysbiosis and its potential association with the development and progression of chronic kidney disease (CKD) has increased substantially in the past 6 years. In parallel, the microbiome field has matured considerably as the importance of host-related and environmental factors is increasingly recognized. Past research output in the context of CKD insufficiently considered the myriad confounding factors that are characteristic of the disease. Gut microbiota-derived metabolites remain an interesting therapeutic target to decrease uraemic (cardio)toxicity. However, future studies on the effect of dietary and biotic interventions will require harmonization of relevant readouts to enable an in-depth understanding of the underlying beneficial mechanisms. High-quality standards throughout the entire microbiome analysis workflow are also of utmost importance to obtain reliable and reproducible results. Importantly, investigating the relative composition and abundance of gut bacteria, and their potential association with plasma uraemic toxins levels is not sufficient. As in other fields, the time has come to move towards in-depth quantitative and functional exploration of the patient's gut microbiome by relying on confounder-controlled quantitative microbial profiling, shotgun metagenomics and in vitro simulations of microorganism–microorganism and host–microorganism interactions. This step is crucial to enable the rational selection and monitoring of dietary and biotic intervention strategies that can be deployed as a personalized intervention in CKD.

Sections

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Key points

- Current kidney replacement therapies are insufficient for the removal of protein-bound uraemic toxins. New therapies or interventions need to be explored to reduce the accumulation of uraemic toxins in patients with chronic kidney disease (CKD).
- Research on the composition of the gut microbiome in patients with CKD has been performed for over 10 years, but globally standardized methods have not yet been established and new techniques are being continuously implemented as technologies advance.
- Any microbiome study of patients with CKD should include a clearly defined (control) group without CKD that is matched as closely as possible for age, body mass index, underlying comorbidities and medication use.
- Characteristics and diet of patients with CKD can influence the composition of the microbiome at the time of sampling. Therefore, diet, transit time and medication use must be taken into account when analysing gut microbiome profiles.
- The latest studies on the effect of dietary interventions on gut microbiome composition and improvements in kidney health show the potential of dietary interventions to change gut microbiome composition and uraemic toxin production.
- At present, no intervention strategies with the ability to improve kidney function are available. Protocol standardization and optimization should lead to and accelerate discovery of new methods of intervention that also improve kidney function.

Introduction

Most of both the general public and the medical community are unaware that a considerable proportion of the general population is at risk of developing chronic kidney disease (CKD)[1]. The Global Burden of Disease Study 2017 reported a 9.1% global prevalence of CKD[2]. CKD develops slowly with no clear symptoms in the early stages of the disease. As the disease progresses, myriad symptoms that are often non-specific and difficult to treat (for example, pain, fatigue, cramps and pruritus) arise, and the condition becomes irreversible. When the kidneys fail, the patient relies on kidney replacement therapy (that is, dialysis or kidney transplantation) to stay alive. In 2017, CKD resulted in 1.2 million deaths worldwide. Moreover, CKD is associated with many health complications such as cardiovascular disease (CVD) and infection[3–5].

A link between CKD and cardiovascular (CV) risk is observed very early in the course of kidney disease[4]. When adjusted for traditional CV risk factors, the presence of impaired kidney function (based on estimated glomerular filtration rate (eGFR)) and albuminuria (based on albumin-to-creatinine ratio) leads to a 2–4-fold increase in the risk of CVD[6]. Importantly, in patients with CKD, the retention of uraemic metabolites triggers several pathophysiological processes that contribute to an increased CV risk, including endothelial dysfunction, oxidative stress and cardiac cell dysfunction, and is associated with CV morbidity and mortality[7]. This association explains why the prevention of CVD with standard interventions that often target traditional risk factors, such as high blood pressure, high cholesterol, diabetes and smoking, is insufficient in patients with CKD. Given that the removal of uraemic toxins by dialysis is hampered by their binding to major blood proteins such as albumin[8], there is a pressing need to explore innovative strategies that can decrease CV burden in patients with CKD.

One of the primary goals in CKD therapeutics is to develop treatments that can limit the accumulation of potential uraemic cardiotoxins, preferably early on in the course of the disease, rather than relying exclusively on the removal of accumulated toxins when the kidneys fail. Of note, current evidence for uraemic toxicity is largely based on in vitro experimental data and relatively small association studies in patients with CKD[7,9], so extrapolation to the clinical setting still warrants caution. Recent insights indicate that gut microbial composition is altered in patients with CKD[10,11], which suggests that the intestinal tract and its microbiota are potentially novel intervention targets. Several uraemia-related factors, such as prolonged transit time, dietary restrictions and medication use, can lead to alterations in gut microbiota composition, which lead to changes in the biochemical milieu of the intestine[12]. In addition, the gut microbiota itself contributes to CKD by generating uraemic toxin precursors through the proteolytic fermentation of dietary aromatic amino acids[11]. Inadequate excretion of uraemic toxins in CKD leads to their accumulation, which creates a uraemic milieu that drives further changes in the gut microbiota[13]. Collectively, these insights suggest the existence of a bidirectional relationship between the gut microbiota and the kidneys, collectively referred to as the gut–kidney axis[14].

In this review we aim to set the scene for a new generation of investigations and trials into the discovery of gut-associated therapeutic targets for CKD. We highlight the importance of host-related and environmental factors, potential confounding factors and quality standards throughout the entire microbiome analysis workflow, as well as the steps needed to enable the selection and monitoring of dietary and biotic intervention strategies.

The human gut microbiome in a clinical context

The human gastrointestinal tract harbours a complex microbial ecosystem that comprises bacteria, archaea, small eukaryotes and viruses. This ecosystem reaches its highest density and diversity in the colon and is commonly referred to as the gut microbiota[15]. Although it was long thought that bacterial cells in the colon outnumber human cells by a factor of 10, current estimates suggest that the ratio between the total number of human cells (nucleated and non-nucleated) and bacterial cells is closer to 1:1 (ref.[16]).

Whereas the microbiota refers to the collection of microorganisms present in a tissue or sample, the microbiome refers to all the microbial genomes present within the microbiota of a microniche or sample[15]. The functional potential of the gut microbiome has a pivotal role in human physiological homeostasis and health maintenance. From a metabolic point of view, one of the most important functions of commensal bacteria is the fermentation of dietary fibres and the consequent production of short-chain fatty acids (SCFAs) that contribute to the maintenance of the gastrointestinal barrier and to immune homeostasis[17]. The gut microbiota also has a crucial role in bile salt deconjugation, vitamin production and glucose homeostasis[18].

Factors affecting the gut microbiota

Early life events, such as the mode of delivery, gestational age at birth, and breast-feeding, can influence the colonization of the pristine human gut with its first microbial communities. These early colonizers have been linked to priming of the adult microbiome and, as a consequence,

can have a substantial effect on host metabolism, and the development of the immune and neuroendocrinological systems in adulthood[19]. Several factors are thought to be co-responsible for the further shaping of the gut microbiota. Host-related factors include age, sex, diet, body mass index (BMI) and intestinal transit time. For instance, a study of monozygotic twins showed that higher BMI correlated negatively with the abundance of SCFA-producing bacteria[20]. Moreover, dietary habits can heavily influence microbiota composition[21]. Notably, Western lifestyle habits and their associated diets, which are characterized by poor intake of fruit and vegetables and high consumption of animal fats and proteins, have been linked with a range of chronic diseases, including obesity, type 2 diabetes (T2D) and CVD[22,23].

Stool consistency, which reflects intestinal transit time and stool water content and partially depends on the aforementioned lifestyle habits, is a major factor affecting microbial faecal composition[24]. Low stool consistency indicates a higher water content and shorter intestinal transit time, whereas harder stools reflect low water content and a longer transit time. This transit time is an indicator of the maturation of the host's colonic ecosystem, which involves a successive series of compositional and functional changes as the microbiota matures towards a stable and metabolically adapted state, and co-determines which species are able to proliferate in the intestinal lumen. Higher transit time increases microbial density and promotes a gradual shift from saccharolytic to proteolytic taxa. The maturation of the colonic ecosystem is reflected by the stool consistency as it is associated with a progressive increase in water (re-)absorption in the large intestine, as well as decreased carbohydrate availability and an increase in microbial proteolytic activity[25]. These events can therefore be considered to be a driving force of gut microbiota variation[25,26].

Driven by the nucleic acid sequencing revolution, numerous efforts have been made to define a 'healthy' microbiome[27], but this concept is not straightforward. Lower taxonomic and functional richness and diversity have been proposed as indicators of an 'unhealthy' microbiota[25,28], but others have questioned this oversimplification[25]. Composition and functionality are also clearly important, as demonstrated by the observation that a shift from saccharolytic fermentation to proteolytic fermentation leads to a decrease in SCFAs and increased production of toxic metabolites[25,29]. However, given that this metabolic activity is regulated by transit time and nutrient availability, a healthy or unhealthy microbiome defined by functional activity might be a fluid characteristic that can change over time.

Microbial signatures associated with health and disease often reflect symbiotic host–bacteria interactions, or a disturbance thereof. These interactions can be of a commensal (beneficial to the host), mutual (beneficial to both host and bacteria) or parasitic (detrimental to the host) nature[30]. The net effect of bacteria on the host often depends on crosstalk between different members of the gut microbiota rather than the activity of a single species. In metabolic cross-feeding, for example, one microorganism uses products formed or metabolized by another microorganism. One example is the interaction between the acetate-producing *Bacteroides thetaiotaomicron* and the acetate-converting and butyrate-producing *Faecalibacterium prausnitzii*[31]. Butyrate has been linked to stimulation of mucin synthesis, which in its turn protects the intestinal epithelium[31,32]. Of note, maintenance of the intestinal epithelium is vital to ensure that microbes are confined to the luminal space and to prevent leakage of microbial toxins into the systemic circulation[33]. In addition, compositional or functional imbalances might deprive the host of specific symbiotic effects leading to dysbiosis[30].

Although diet and lifestyle have an important role in gut microbiome disturbances, several other covariates have been linked to microbiome variation. Antibiotic use is recognized as a major trigger of dysbiosis, for example, by causing a reduction in microbial production of SCFAs[30,34,35]. A large cross-sectional metagenomic study of gut microbiota in the Flemish population demonstrated that the use of β-lactam antibiotics is one of the key drivers of microbial composition[24]. The same study also linked the use of other medications, such as laxatives, to changes in the gut microbiome[24].

Gut microbiome studies in CKD

Compared with other disease associations, the potential link between gut microbiota composition and CKD is a relatively recent area of research in the human microbiome field and was first reported in 2013 (ref.[13]). This early research was based on insights from articles published in the 1990s on the effect of uraemia on intestinal permeability and bacterial translocation, as well as new findings showing the presence of endotoxaemia and histological evidence of chronic enterocolitis in patients with kidney failure, and the disruption of tight junction molecules in uraemic rats[36,37]. Using a phylogenetic microarray approach, abundant differences in 190 bacterial operational taxonomic units were reported between a small cohort of 24 patients with kidney failure and a control group of 12 healthy persons[13]. Since then, over 400 studies have reported CKD-associated changes in the composition of the human gut microbiome and how these alterations might affect disease progression and the development of comorbidities. For retrospective interpretation and comparative purposes, it is important to note that methodological approaches have gradually shifted from targeted quantitative polymerase chain reaction and 16S ribosomal RNA amplicon sequencing, to untargeted whole-genome shotgun metagenomics (Box 1 and Table 1).

Patient factors and the gut microbiome in CKD

Myriad covariates affecting the gut microbiome composition are commonly present in patients with CKD, including multidrug therapy, comorbidities, uraemic milieu, low-fibre diet, limited mobility, constipation and ageing[38]. However, in contrast to routinely recorded parameters such as age, eGFR and comorbidities, factors known to affect microbial composition such as medication use, exact dietary records in the days leading up to sampling and stool consistency were often disregarded or ignored in CKD microbiome studies (Table 1). The lack of such crucial information precludes an appropriate deconfounder analysis and might lead to biased microbial signatures or association patterns when comparing patient and control groups. Notably, several common comorbidities of patients with CKD such as T2D[39], hypertension[40], CVD[41] and depression[42] have been associated with dysbiotic gut microbiome signatures. However, cause and consequence of gut dysbiosis are often unclear and the aetiology of CKD is rarely taken into account. Microbial similarity is lower between dizygotic twins than between monozygotic twins, which suggests that host genetics also influences the gut microbial phenotype, with variable effects across taxa[43]. Although genes and gene polymorphisms underlying hereditary kidney diseases such as autosomal-dominant polycystic kidney disease and cystinosis might thus potentially affect the gut microbiome, to our knowledge this has not been studied. In the past few years, additional associations between host genetics and microbiome composition have been described, but the effect size is smaller than previously presumed[44]. Moreover, heterogeneity between studies makes it challenging to determine an exact contribution of the host

genetics on the gut microbiome composition and it remains difficult to separate the genetic from the environmental effect.

With regard to multidrug therapy, in a cohort of 2,173 European residents, frequently prescribed drugs, which are also prescribed to patients with CKD and often taken in combination (such as statins, β-blockers, metformin, aspirin, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers), explained more of the variation in microbiome composition than disease groups (for example, hypertension, dyslipidaemia and T2D) or any other parameters[45]. Importantly, drug effects are often dose dependent, which is rarely taken into account in microbiome studies.

The incidence of CKD is higher in the ageing population, increasing from ± 50 per million population (pmp) in individuals aged 45–64 years, to ± 350 pmp among those aged 65–74 years and ≥500 pmp in individuals >75 years of age[46]. Ageing coincides with a decrease in α-diversity of the gut microbiome and an increased abundance of the genera *Bacteroides, Lactobacillus* and *Escherichia*. Collectively, these changes highlight the importance of selecting age-matched control groups in clinical study designs[47]. Moreover, older age and disease severity often affect the ability to perform physical activity, which correlates positively with survival rates in patients with CKD not receiving dialysis[48]. A 2022 systematic review concluded that increased exercise or physical activity in older adults (50–98 years old) has a beneficial effect on gut microbiome composition[49], potentially resulting in a healthier intestinal milieu with increased levels of the SCFA butyrate after a 24-week intervention comprising CV and resistance exercise[50]. Butyrate contributes to improved mucus production and tight junction expression and/or the facilitation of its assembly[51,52]. These effects are especially relevant for patients with CKD as they might help to prevent the translocation of bacterial derivatives and reduce low-grade inflammation. Increased physical activity might also decrease transit time. As mentioned earlier, transit time is an important covariate of gut microbiome composition that can, when prolonged, induce an upstream expansion of proteolytic bacteria as a large parts of the colon become deprived of carbohydrates[53]. Compared with healthy controls and patients on peritoneal dialysis, patients on haemodialysis have a significantly longer mean colonic transit time[54]. Of note, constipation has been associated with CV events in postmenopausal women[55] and with an increased CKD risk in US veterans[56].

The diet of patients with CKD can also greatly affect the gut microbiome. To limit serum potassium levels, with the aim of reducing the risk of CV events, patients with CKD are often advised to reduce fruit and vegetable intake, resulting in a reduced fibre intake, which unfavourably affects the nutrient supply to saccharolytic bacteria. Diet might also be a primary driver of faecal metabolome composition as the faecal metabolome of patients receiving dialysis could not be distinguished from that of their household contacts[57]. Finally, exacerbation of the uraemic milieu as CKD progresses causes an influx of urea and uric acid into the intestinal lumen via the enterohepatic cycle, which significantly increases the abundance of bacterial species that express urease, uricase and enzymes that generate indole and *p*-cresol, while decreasing butyrate-producing species (Fig. 1). Increased plasma levels of uraemic toxins were also identified as covariates of microbiome composition[10,38].

Collectively, these findings indicate that future studies of gut microbiota composition and function in CKD will benefit from the collection of as many relevant covariates as possible to ensure the biological and clinical relevance of any uncovered host–microbiota correlations and associations.

Box 1

16S rRNA gene amplicon and shotgun sequencing technologies

Investigations of the microbial composition within complex samples have long been performed using next-generation amplicon sequencing based on the 16S ribosomal RNA (rRNA) gene[165]. This gene is highly conserved and present in most bacterial genomes; therefore, this approach is very useful for simple taxonomic identifications[166]. However, given the drop in sequencing costs, 16S rRNA gene amplicon sequencing is slowly being superseded by shotgun sequencing (Table 3).

Shotgun sequencing covers all the genetic content in a sample rather than targeting specific genes such as that encoding 16S rRNA. Consequently, this technique provides high-resolution data and enables culture-independent assessment of microbial communities down to species and strain level[153,167]. Shotgun sequencing also supports the assembly of gene catalogues, thus allowing investigations into the functional potential of metabolically active bacteria[153]. Illumina sequencing is the most widely used technology for metagenomic profiling as it enables high read accuracy while keeping the sequencing cost for large cohort studies relatively low[168]. For example, the Illumina GAIIx platform was used in the Human Microbiome Project to characterize various human microbiomes of healthy participants living in industrialized countries[127]. However, one of the major drawbacks of Illumina technology is the relatively short read length, which can complicate downstream data processing[169].

Targeting uraemic metabolites in CKD

In contrast to healthy populations, in whom protein assimilation (that is, protein digestion, metabolism and absorption) occurs predominantly in the small intestine, in patients with CKD (regardless of whether or not they receive dialysis), protein assimilation is impaired and levels of undigested or unabsorbed proteins entering the colon are increased[58,59]. This alteration creates nutritional conditions that favour proteolytic bacterial species[60]. In the distal part of the colon, dietary and endogenous amino acids such as tyrosine, phenylalanine and tryptophan are used for bacterial growth or are further metabolized into various end-metabolites such as phenols and indoles (Fig. 1). Although early in vitro evidence had already shown that gut bacteria could produce uraemic toxin precursors[61], in 2011 a study demonstrated the colonic origin of well-known uraemic (cardio)toxins such as pCS and IxS by comparing their concentrations in plasma of patients receiving haemodialysis with and without colectomy[62]. Subsequent untargeted metabolomics using liquid chromatography coupled to mass spectrometry identified 46 colon-derived uraemic metabolites[63]. In addition to metabolomics, other -omics approaches such as proteomics and peptidomics can help to explore the gut–kidney axis and to identify targets for intervention in CKD in an untargeted manner[64].

Table 1 | Microbial profiling methods and data collection in major gut microbiome studies in CKD

CKD stage[a]	CKD cohort (non-CKD control)	Profiling method	Data collected and published								Ref.
			Age	eGFR	Diabetes	Co-morbidity	Dietary recall	Med	ITT	Activity level	
5 (HD)	24 (12)	16S rRNA	Yes	NA	Yes	NA	NA	NA	NA	NA	13
3, 4	31 (NA)	16S rRNA	Yes	Yes	NA	Yes	NA	NA	NA	NA	95
5 (HD)	10 (NA)	16S rRNA	Yes	NA	Yes	Yes	NA	NA	NA	NA	132
4, 5[b]	20 (NA)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	133
5 (PD)	20 (NA)	16S rDNA	Yes	NA	Yes	NA	Yes	Yes	NA	NA	134
5 (HD)	20 (NA)	16S rRNA	Yes	NA	Yes	NA	NA	NA	NA	NA	135
3, 4	16 (NA)	16S rRNA	Yes	Yes	NA	NA	Yes	Yes	NA	NA	136
5, 5 (HD)	53 (69)	16S rRNA	Yes	Yes	NA	NA	NA	Yes	NA	NA	137
4, 5[b]	20 (20)	16S rRNA	Yes	Yes	Yes	NA	NA	Yes	NA	NA	138
5 (HD)	62 (NA)	MALDI-TOF	Yes	NA	NA	NA	NA	NA	NA	NA	139
5 (HD)	18 (20)	16S rRNA	Yes	NA	NA	NA	NA	NA	NA	NA	140
1–5	110 (210)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	141
1–5 (PD)	168 (30)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	142
1–5	72 (20)	Shotgun	Yes	Yes	Yes	Yes	NA	NA	NA	NA	143
5 (HD)	85 (NA)	16S rRNA	Yes	NA	Yes	Yes	Yes	Yes	Yes	NA	144
5 (PD)	15 (NA)	16S rRNA	Yes	Yes	Yes	NA	Yes	Yes	NA	Yes	94
3–5	80 (78)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	145
5 (HD)	223 (69)	Shotgun	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	146
1–5	111 (NA)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes	10
1, 2[c]	35 (35)	16S rRNA	Yes	NA	Yes	NA	NA	NA	NA	NA	147
5 (HD)	20 (NA)	16S rRNA	Yes	NA	Yes	Yes	Yes	Yes	NA	NA	148
1–5[b]	95 (NA)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	149
3–5	25[d] (34)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	150
1–5	100 (100)	16S rRNA	Yes	Yes	Yes	Yes	NA	Yes	NA	NA	151
5 (HD)	12 (NA)	16S rRNA	Yes	NA	Yes	Yes	NA	NA	NA	NA	152
3	37 (74)	Shotgun	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	153
3–5	59 (NA)	16S rRNA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	93
5	36 (NA)	16S rRNA	Yes	Yes	Yes	NA	Yes	Yes	NA	NA	154
All studies			28/28	19/28	23/28	17/28	9/28	19/28	3/28	2/28	

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HD, haemodialysis; ITT, intestinal transit time; MALDI-TOF, matrix-assisted laser desorption ionization–time-of-flight; Med, medications; NA, not available; PD, peritoneal dialysis; rRNA, ribosomal RNA. [a]Patients not receiving dialysis unless otherwise indicated. [b]Cohort of patients with diabetes. [c]Cohort of patients with idiopathic nephrotic syndrome. [d]Colonic dialysis cohort.

From a biotherapeutic point of view, such interventions should be aimed at restoring eubiosis, decreasing levels of uraemic toxins and improving the outcomes of patients with CKD. The association between increased levels of uraemic toxins of colonic origin and an increased risk of CVD and the progression of CKD[9,65] has been widely discussed but, as long as the accumulation of the respective uraemic toxins cannot be prevented or levels cannot be decreased significantly in CKD, causality cannot be proven.

Different approaches are currently being explored to prevent or decrease the accumulation of uraemic toxins, including dietary and biotic interventions, and the use of transporter inhibitors[66] and oral adsorbents that bind uraemic toxin precursors in the intestine, thereby preventing their absorption. One example of these sorbents is the orally administered AST-120, which has been suggested to delay progression of CKD and initiation of dialysis[67], although the most recent randomized controlled trials did not confirm this hypothesized impact on the progression of CKD[68,69].

Interventions in patients with CKD should focus not only on (precursors of) uraemic toxins but also on microbial metabolites with beneficial effects on human metabolism, the epithelial barrier and the immune system, and that are either decreased (butyrate) or show a decreasing trend (acetate and propionate) in CKD[12,70,71]. By preserving or restoring the intestinal barrier function, both active and passive transport of bacterial derivatives and metabolites could be positively

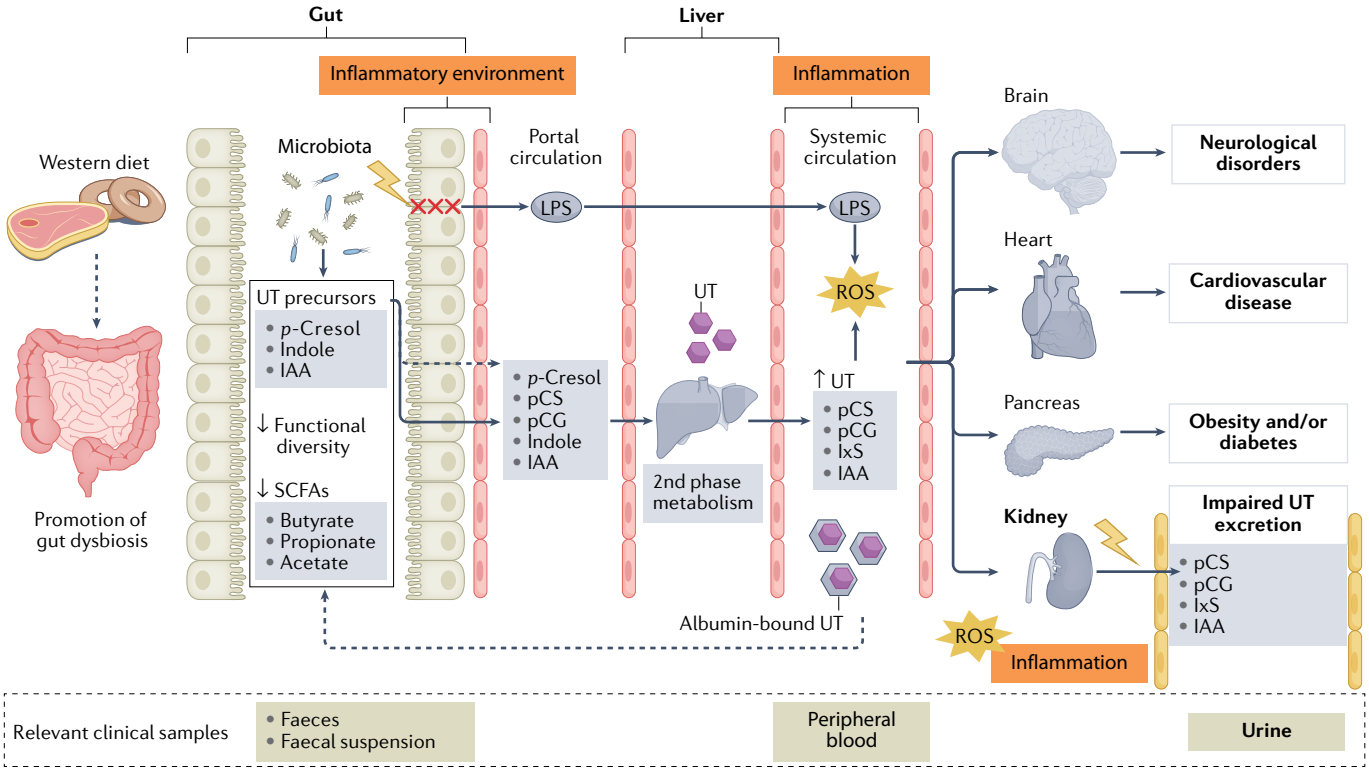

Fig. 1 | The gut–liver–kidney axis. Western lifestyle and its associated diet (low in fruit and vegetables, and rich in animal fats and proteins) have been linked to chronic diseases such as obesity, type 2 diabetes and cardiovascular diseases. These conditions are characterized by decreased functional microbial diversity and an increased inflammatory environment. Dysbiosis of the gut microbiome has also been linked to neurological disorders and depression. Under normal non-dysbiotic conditions, myriad end-products of the gut microbiota metabolism (for example, *p*-cresol, indole and indole acetic acid (IAA)) are absorbed towards the portal circulation through the intestinal barrier and modified via second-phase metabolization, especially in the liver. The downstream metabolites generated by this process (for example, *p*-cresyl sulfate (pCS), *p*-cresyl glucuronide (pCG) and indoxyl sulfate (IxS)) are released into the systemic circulation and some of them bind to albumin. Protein-bound solutes rely on kidney tubular secretion for clearance into the urine. Consequently, when kidney function deteriorates, metabolites of colonic origin accumulate in the blood, some of which have deleterious biological effects and are termed uraemic toxins (UT). High plasma levels of UT contribute to organ toxicity and might influence gut microbiota composition and function, promoting an increase in UT-producing bacterial species and a decrease in short-chain fatty acid (SCFA)-producing bacteria. This imbalance is deleterious for intestinal barrier function as it contributes to disruption of the mucus layer and decreased expression of tight junction proteins. Consequent increases in intestinal permeability might cause leakage of bacterial derivatives such as lipopolysaccharide (LPS), which could contribute to local and systemic inflammation, and oxidative stress. To what extent the generation of UT precursors and leakage through the intestinal barrier contribute to increased circulating levels of UT, which is associated with the systemic inflammation and oxidative stress (due to production of reactive oxygen species (ROS)) observed in chronic kidney disease remains to be clarified.

affected, thereby decreasing low-grade inflammation and the load of uraemic metabolites in CKD. Finally, it should be emphasized that not all metabolites in uraemic toxin-generating pathways are necessarily toxic at their site of origin. For instance, although the tryptophan-derived metabolites IxS, kynurenine and kynurenic acid are toxic throughout different compartments of the body, metabolites of the same pathway such as indole and indole-3-propionic acid mainly exert beneficial effects (for example, on the intestinal barrier)[72]. This stratification in site-specific toxicity suggests that restoring homeostasis in CKD might be a complex balancing act.

Dietary interventions in CKD

A range of dietary guidelines have been investigated as potential therapeutic strategies for CKD including Mediterranean diets (rich in vegetables, nuts, legumes, fruits and whole grains), plant-based diets and low-protein diets (LPDs; reduced dietary protein intake but avoiding the complete absence of protein)[73–75]. These diets are aimed at decreasing bacterial proteolytic fermentation by reducing the high protein consumption that is typically associated with Western diets[73]. As a result, inflammatory responses and uraemic toxin production are expected to decrease, with the expectation that kidney function decline might be slowed down and CV risks might be reduced.

Several studies have investigated whether LPDs lead to gut microbiome changes in individuals with CKD. One meta-analysis concluded that overall gut microbial diversity did not differ substantially between individuals with CKD on an LPD compared with patients on a normal protein diet or healthy controls (on an LPD or a normal protein diet). Moreover, an LPD did not change uraemic toxin levels or kidney function (based on eGFR and blood urea nitrogen) in patients with CKD compared with a normal protein diet[76]. Of note, the abundance of various SCFA-producing species such as *Roseburia faecis* was higher in the patients with CKD on an LPD compared with controls, but these

changes seemed insufficient to alter any of the investigated metabolic or clinical outputs[76].

Very few studies have investigated the potential link between plant-based diets, uraemic toxin production and the gut microbiota. One clinical trial concluded that the quality of diet and food selection might potentially affect uraemic metabolite synthesis and gut microbiota composition in adults receiving haemodialysis[75]. These early observations point towards the potential of dietary gut microbiota modulation but warrant further and more in-depth intervention studies to fully clarify the effects of LPD or plant-based diets on diet–microbiome–host interactions in patients with CKD.

Biotic interventions in patients with CKD

As discussed above, the CKD-associated impairment of protein assimilation has encouraged dietary interventions characterized by restricted protein intake to reduce the concentration of protein substrates potentially available for uraemic toxin synthesis[77]. However, biotic interventions might normalize CKD-induced dysbiosis and reduce the activity or presence of proteolytic bacterial species while increasing the activity or presence of saccharolytic bacterial species.

Glossary

Bacterial translocation
The passage of bacteria from the gastrointestinal tract into systemic circulation.

Bristol Stool Form Scale
Diagnostic medical tool used to classify faeces into seven groups based on shape and consistency.

Cardiotoxins
Toxins that have effects on the heart and vessels that lead to undesirable outcomes.

Covariates
A study participant variable that might influence the results of what is being studied.

Deconfounder analysis
An analysis that is aimed at identifying which variables indirectly influence the outcome of a study and might thus introduce a confounding bias.

Dysbiosis
A compositional or functional imbalance of the gut microbiota linked to a disease state.

Functional analyses
Analyses of the metabolic potential of the gut microbiome.

Gut–kidney axis
Interplay between the gut (and the microbial community it accommodates) and the kidneys, mediated by endogenous transport mechanisms and metabolism-dependent pathways.

Microniche
A bacterial habitat offering specific conditions for optimal proliferation of one or more specific species.

Proteolytic fermentation
Bacterial degradation of (dietary) protein with production of (mostly) detrimental metabolites such as urea.

Quantitative microbial profiling
Absolute quantification of microbial taxa in complex samples.

Relative microbial profiling
Estimation of the relative frequency of microbial taxa in complex samples.

Saccharolytic fermentation
Bacterial degradation of (dietary) non-digestible carbohydrate with production of SCFAs.

Structured diet history method
Detailed assessment of daily food intake.

Effects on kidney function, uraemic toxin levels and inflammatory markers in CKD. In addition to dietary changes, the therapeutic potential of biotic interventions has been investigated in CKD. Here, the term 'biotics' includes prebiotics, probiotics as well as synbiotics. Prebiotics are non-digestible substrates that are selectively used by host microorganisms and confer a health benefit (for example, fibre compounds such as fructo- and galacto-oligosaccharides, inulin and resistant starch)[78]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (for example, specific strains of *Bifidobacterium*, *Lactobacillus* and *Streptococcus* have been used in probiotic products)[79]. Synbiotics represent a mixture of live microorganisms and prebiotics[80].

In general, meta-analyses of studies in patients with CKD have not reported significant changes in primary outcomes such as kidney function (based, for example, on eGFR, serum creatinine, blood urea nitrogen and albumin) or CV events[81–85]. By contrast, most meta-analyses reported that biotics could modulate levels of uraemic toxins in body fluids and of circulating inflammatory markers such as C-reactive protein (CRP) and/or pro-inflammatory cytokines (for example, IL-6)[81–89], but some results are conflicting. For example, one meta-analysis found that pre-, pro- and synbiotic interventions were associated with a significant decrease in pCS, CRP and IL-6 (ref.[88]), whereas another reported that all interventions could reduce CRP levels but only prebiotic interventions reduced IL-6 levels, without any effects on TNF or levels of uraemic toxins (pCS and IxS)[90]. Several meta-analyses that examined changes in uraemic toxins specifically indicated a decrease in uraemic toxin levels (mainly IxS and pCS) in patients with CKD stages 3–5 not on dialysis[83,87,89], whereas others indicated little or no change in uraemic toxin levels[81,84,86]. This broad range of clinical outcomes might not only reflect differences in patient characteristics, study sample size, baseline kidney function status and follow-up time of the randomized controlled trials (RCTs) but also emphasizes that biotics are highly heterogeneous in terms of individual strain properties (probiotics), biological origin and chemical structure (prebiotics), as well as biological activity. Moreover, comparative data analyses are further complicated by the fact that each meta-analysis defined different inclusion and exclusion criteria, and focused on different primary and secondary outcomes. Collectively, these observations call for better harmonization of intervention study designs, including a list of common kidney function and microbiome-related readouts, which will enhance the ability to compare different studies for a deeper and more extensive understanding of the effect of biotics on CKD.

Effects on gut dysbiosis in CKD. In addition to their potential effect on uraemic toxin levels and their anti-inflammatory potential, biotics can also be employed therapeutically to enhance recovery from dysbiosis[91]. The bidirectional relationship between gut dysbiosis and disease progression is not limited to CKD, but also has a role in CKD-related complications such as CVD, and mineral and bone disorders[92]. Although an increasing number of studies are starting to incorporate microbiome profiling, only a limited number of RCTs have used this approach to evaluate potential intervention effects (Table 2). Out of five clinical trials, four did not reveal significant changes in kidney function after pro-, pre- or synbiotic intervention[90,93–95]; one study reported a reduction in eGFR and an increase in serum creatinine after long-term synbiotic therapy in patients with CKD stage 3 or 4 not receiving dialysis[87]. However, in terms of microbiome-associated changes, all five studies identified taxa that increased or decreased in abundance after the biotic regimen[82,87,93–95] (Table 2). However, only three trials measured

lower uraemic toxin levels in faeces or serum in addition to changes in microbial taxa[82,93,95], whereas the remaining two trials reported no changes in uraemic toxin levels after biotic intervention[87,94].

Despite showing the potential to affect both the microbiome composition and uraemic toxin levels positively, the limited number of published reports indicates that biotic interventions in cohorts with CKD are still in their infancy. Moreover, the available studies differ substantially in the type of intervention (synbiotics versus prebiotics alone), type of study (RCT versus crossover study), study duration (short-term versus long-term) and patient groups (moderate CKD versus kidney failure, and dialysis or non-dialysis treatment). By contrast, all study designs had a relatively small sample size and study duration in common, and these two factors are known to limit statistical power for detection of changes in primary outcomes such as kidney function or CV events[82,87,93–95]. These limitations also call for future studies to be designed with a longer follow-up of larger, adequately powered and placebo-controlled randomized cohorts. Furthermore, confounding factors such as antibiotic use might be very difficult to disentangle from the effects of the biotic intervention. For example, in one study, decreases in pCS and IxS concentrations following biotic intervention were more pronounced in patients who did not receive antibiotics. The researchers suggested that the antimicrobial action exerted against the administered probiotics was relatively stronger than that against IxS-producing bacteria, but a detailed investigation was not possible owing to the small sample size and the variability in the antibiotics used by study participants[95]. Of note, most other studies have excluded patients taking antibiotics[82,87,93,94].

Biotic interventions are promising — they are cheap compared with drug therapy for co-morbidities associated with CKD, as well as kidney replacement therapy, have very few side effects and are well tolerated. In addition to the handful of published studies, 10 new clinical trials are currently registered to assess the effect of biotic intervention on both the gut microbiome and uraemic toxin production[96–105]. The majority of these studies are RCTs that are investigating the use of prebiotics (for example, inulin, resistant starch and oligofructoses) or probiotics (specific strains of *Bifidobacterium*, *Lactobacillus* and/or *Streptococcus*) in patients with CKD (ranging from CKD stage 3 to kidney failure) for ~6 months. Collectively, these studies offer interesting prospects to advance the current knowledge on the potential of biotics to modulate the gut–kidney axis in CKD.

Combining biotic and dietary interventions might maximize the therapeutic potential of these approaches. For instance, in patients with CKD on an LPD, and compared with placebo, probiotic strains of *Bifidobacterium longum* and *Lactobacillus reuteri* had additional beneficial effects to those of an LPD on the control and modulation of microbiota-derived and proatherogenic toxins, while also increasing survival of patients not receiving dialysis. Moreover, a reduction of antihypertensive and diuretic medications was possible in the probiotics group[106].

Faecal microbiota transplantation in CKD

In addition to dietary and biotic interventions, faecal microbiota transplantation (FMT), which involves faecal infusion from a healthy donor to the gastrointestinal tract of a recipient patient, might offer an additional strategy to modulate the gut microbiota of patients with CKD.

Currently, FMT has only been accepted as a safe and promising treatment option in severe cases of recurrent *Clostridium difficile* infection[107], with an efficiency rate >80%. To date, the potential of FMT as a

Table 2 | Biotic intervention studies in patients with CKD

Study design	Study duration (weeks)	CKD stage[a]	*N*	Supplementation	Uraemic toxin changes	Taxa changes post-intervention	Other remarks	Ref.
RCT, SC, DBP	18	4, 5	37	9 bacterial strains across *Bifidobacterium*, *Lactobacillus* and *Streptococcus* genera combined with inulin[b], FOS[b], GOS[b]	↓pCS ↓IxS[b]	↑*Bifidobacterium* spp. ↑Lachnospiraceae ↑*Faecalibacterium* spp.[c] ↓Clostridiales ↓Ruminococcaceae	↑Albuminuria	95
RCT, SC, DBP	26	5 (HD)	45	*Bifidobacterium longum* NQ1501, *Lactobacillus acidophilus* YIT2004 *Enterococcus faecalis* YIT0072	↓pCS[d]	↑Bacteroidaceae ↑Enterococcaceae ↓Ruminococcaceae ↓Halomonadaceae ↓Erysipelotrichaceae ↓Peptostreptococcaceae ↓Clostridiales family XIII	NA	82
RCT, DBP	38	5 (PD)	21	ITF[b]	None	↓Abundance of indole-generating species	↓Intestinal pH	94
RCT, DBP	52	3, 4	56	9 strains across *Bifidobacterium*, *Lactobacillus*, *Streptococcus* genera combined with HRSF[b]	None	↑*Bifidobacterium* spp. ↑*Blautia* spp.	↑Creatinine ↑eGFR	87
RCT, SC, SB	14	3, 4	59	β-glucan fibre[b]	↓Free pCS ↓fFee IxS ↓pCG	Shift from *Bacteroides* 2 enterotype to *Prevotella* enterotype	↓LDL cholesterol	93

DBP, double-blind placebo-controlled trial; eGFR, estimated glomerular filtration rate; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; HD, haemodialysis; HRSF, high-resistant starch fibre; ITF, inulin-type fructans; IxS, indoxyl sulfate; LDL, low-density lipoprotein; SB, single-blind trial; N, number of people enrolled in the trial; NA, not applicable; PD, peritoneal dialysis; pCG, *p*-cresyl glucuronide; pCS, *p*-cresyl sulfate; RCT, randomized controlled trial; SC, single centre. [a]Patients not receiving dialysis unless otherwise indicated. [b]Probiotic supplementation. [c]In participants not receiving antibiotics. [d]In participants without diabetes.

Box 2

Indispensable metadata and CKD-related metadata for microbiome analysis

Patient and sample characteristics and survey data:
- Patient ID
- Age
- Sex
- Disease stage (if applicable)
- Geospatial location
- Date of collection of informed consent
- Specific diet (for example, gluten-free)
- Pets
- Dental records
- Birth type
- Breastfeeding
- Physical activity
- Sample ID
- Sample quantity
- Time and date of sampling
- Time since last defaecation
- Bristol Stool Form Scale
- Diarrhoea or constipation

Patient anthropometry and medical data
- Body weight
- Height
- Waist circumference
- Body mass index
- Blood pressure
- Heart rate
- Prescribed medication
- Over-the-counter medication
- Vitamins and supplements
- Antibiotics in last 6 months
- Medical conditions
- Current infections
- Allergies
- Pregnancy (if applicable)
- Menstruation (if applicable)
- Vaccination history
- Surgical history
- Smoking status

Blood panel: complete blood count
- Leukocytes (including differentiated)
- Haemoglobin
- Haematocrit
- Erythrocytes
- Thrombocytes
- Mean corpuscular volume
- Mean corpuscular haemoglobin

Blood panel: comprehensive metabolic panel
- Total protein
- Albumin
- Aspartate aminotransferase
- Alanine aminotransferase
- Gamma-glutamyl transpeptidase
- Bilirubin
- Urea
- Creatinine
- Glucose
- Haemoglobin-A_{1C}
- Sodium
- Potassium
- Calcium
- Chlorine
- Magnesium
- Phosphorus
- Bicarbonate
- Ferritin
- Cobalamin

Blood panel: lipids
- Triglycerides
- Total cholesterol
- High-density lipoprotein cholesterol
- Low-density lipoprotein cholesterol

Blood panel: others
- C-reactive protein
- Uric acid
- Alpha 1 globulin
- Alpha 2 globulin
- Beta globulin
- Gamma globulin
- Creatinine kinase
- Uraemic metabolites of colonic origin[a]

Urinalysis
- Creatinine
- Phosphorus
- Uric acid
- Total protein
- Uraemic metabolites of colonic origin[a]

Faecal sample data
- Sample weight
- Wet weight
- Dry weight
- Bacterial cell count (total or intact)
- Faecal calprotectin
- Metabolites of proteolytic or carbohydrate fermentation[a]

Dialysis therapy data[a]
- Dialysis type
- Dialysis time
- Residual kidney function
- Blood flow rate
- Dialysate flow rate
- Dialysis efficiency
- Ultrafiltration rate

[a]CKD specific

valuable approach to correcting uraemic toxin levels and preserving kidney function in patients with CKD has not been investigated. In mice with CKD, FMT improved p-cresol-derived uraemic toxin accumulation and reduced CKD complications by having a beneficial effect on gut microbiota (increased α-diversity)[108]. Clearly, further studies are needed to investigate the effect of donor and recipient characteristics, duration and dosage on the efficacy of FMT before this approach can become a therapeutic option in patients with CKD.

Guidelines for human gut microbiome studies

Studies linking the composition and function of the gut microbiota to disease parameters can only provide meaningful clinical predictions if they meet basic requirements in terms of cohort selection and metadata collection. For both patient and (healthy) control groups, recruitment and selection should capture a sufficiently powered and representative population size to illustrate the disease phenotype and enable the investigation of correlations and associations between

groups and individuals[24,109,110]. Cohort selection should therefore be based on strict inclusion and exclusion criteria to rule out clinical confounders known to perturb the microbiota[24]. A clinical cohort selection must follow diagnosis guidelines for the condition studied. In CKD for instance, study cohorts should ideally represent all stages of the disease as defined by the Kidney Disease Outcomes Quality Initiative and Kidney Disease Improving Global Outcome guidelines[1,10,111,112].

The most common exclusion criteria include comorbidities, acute illness, abnormally high or low BMI, the use of systemic antimicrobials in the last 6 months, treatment with cytotoxic medications, unstable dietary habits and a history of recent procedures that affect the gastrointestinal tract directly, such as colonoscopy[24,110,113]. Likewise, the selection of a healthy control cohort should follow stringent exclusion criteria. To minimize the effect of intrinsic and extrinsic factors on the supposed cause and effect, the healthy group must be ideally matched for potential confounders such as geographical location (for example, urban versus non-urban locations), BMI and age category[114]. As the gut microbiome composition changes substantially during childhood, adolescents should be studied as a separate cohort[115,116]. Finally, for diagnostics-based studies of disease-specific characteristics, the definition of a healthy control should be based on the clinical condition in question rather than the overall health status of the participant[11].

Following participant selection and consented sample collection, extensive consented metadata that capture as many relevant confounding factors as possible should be collected to aid the unbiased interpretation of microbiome readouts at an individual and population level (Box 2). All metadata parameters must follow the International System of Units system, adhere to the FAIR principles (that is, findability, accessibility, interoperability and reusability) and respect the privacy of the participant in accordance with data protection laws, such as the General Data Protection Regulation. General parameters regarding samples and donors, such as sample collection date, donor identification number, age, anthropometry and sex, usually become the first set of data collected[117]. Faecal sample-specific parameters, including time since last defaecation and Bristol Stool Form Scale, which allows an estimation of intestinal transit time, can be self-assessed[24,110]. Lifestyle parameters, such as smoking status, and medication can also be recorded through electronic questionnaire systems[110].

Dietary habits and consumption of certain food groups can be assessed and quantified by dietitians using an open-ended, structured

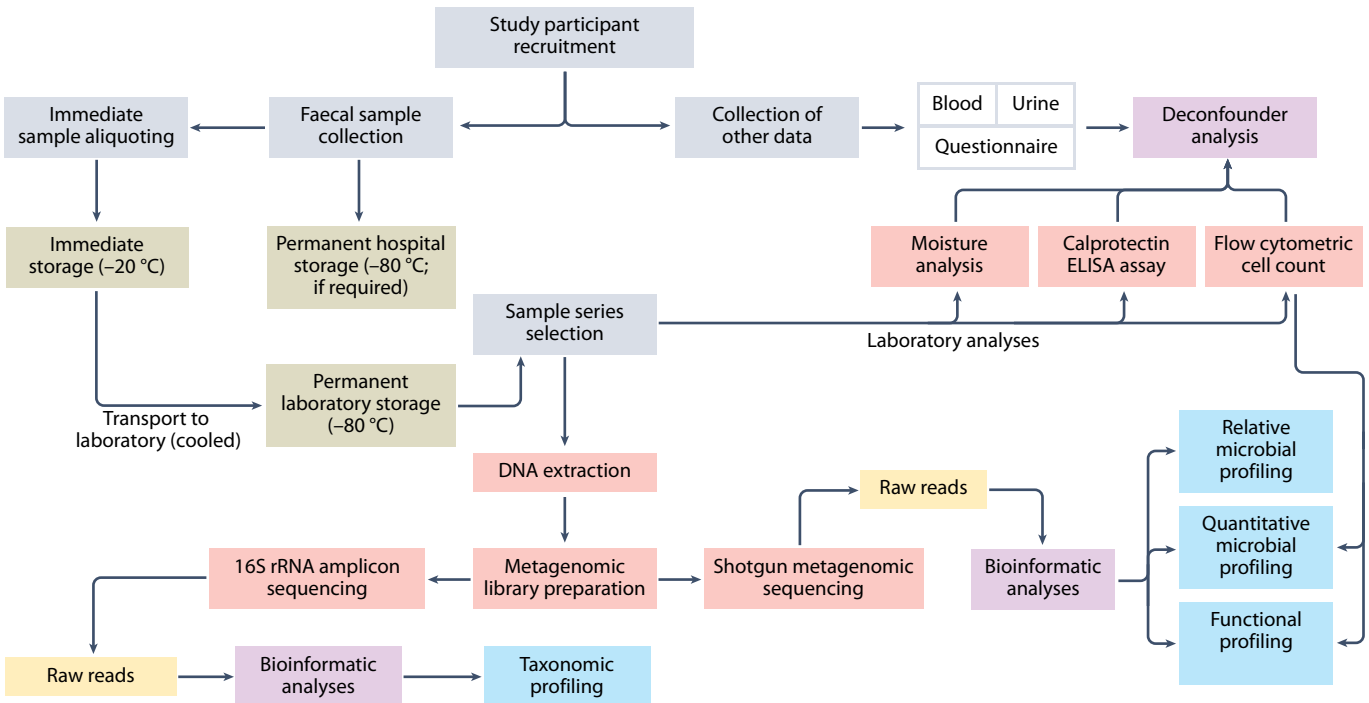

Fig. 2 | Sample handling pipeline for gut microbiome studies. All samples begin with a study participant as well as collection of their faecal, blood and urine samples, as well as medical and lifestyle questionnaires. To minimize practical inconveniences for study participants, faecal collection kits are preferably designed for home sampling, immediate sample aliquoting and storage at −20 °C until transfer to the laboratory or the hospital facility, where they should be stored at −80 °C until further processing. Storage at such low temperatures also allows the use of aliquoted samples in any future follow-up analyses, if necessary. Samples stored at −80 °C for a period of up to 5 years yield a similar microbiome composition to samples analysed shortly after collection, provided that the same protocol is followed[164]. Next, faecal microbiome DNA is extracted and prepared for library construction. To minimize the introduction of batch effects during the extraction process, all faecal samples should be processed within a similar time frame using the same protocol to avoid day-to-day variability[122]. Depending on the aim of the study, various profiling approaches can be taken post-library preparation. Basic identification and comparison of bacterial taxa present in samples is commonly performed using the cost-effective 16S ribosomal RNA (rRNA) gene sequencing. However, if a better taxonomic resolution and/or a functional profile is needed, then shotgun metagenomic sequencing should be used. This method enables culture-independent assessment of microbial communities, down to species and strain level, and supports the assembly of gene catalogues, which enable the investigation of bacterial-derived metabolites and their functions. Sampling of the human gut microbiome often results in uneven sampling depth, which can affect results when analysing unevenly distributed or diverse microbiomes; these data can be corrected by integrating bacterial cell counts obtained by flow cytometry to yield quantitative rather than relative profiles.

Table 3 | Comparison of selected sequencing platforms most commonly used in microbiome research

Technology	Method	Platform	Run time (h)	Max output (Gb)	Max reads per run	Average read length (bp)	Instrument cost[b]	Error rate (%)	Refs.
Illumina	Sequencing by synthesis	MiniSeq	4–24	7.5	25,000,000	2×150	€	0.4	155
		MiSeq	4–55	15	25,000,000	2×300	€€	0.6	156
		NextSeq 550	12–30	120	400,000,000	2×150	€€€	0.6	157
		HiSeq 4000	24–84	1,500	500,000,000	2×150	[c]	0.1	158
		HiSeq X ten	<84	1,800	6,000,000,000	2×150	[c]	0.09	159
		NovaSeq 6000	13–44	6,000	6,000,000,000	2×250	Available by request	0.1	160
PacBio	Single molecule, real-time sequencing	RS II	0.5–4	10	55,000[a]	15,000	[c]	10–15	161
		Sequel I	<20	10	365,000[a]	15,000	Available by request	10–15	162
		Sequel II	<30	500	4,000,000,000[a]	15,000	Available by request	10–15	162
Nanopore	Strand sequencing	MinION	<72	50	No max	4,000,000	€	13	162,163
		GridION	<72	250	No max	4,000,000	€	13	162,163
		PromethION24	<72	290	No max	4,000,000	€€€	13	162,163

€, cost range < €50,000; €€, cost range €50,000–100,000; €€€, cost range > €100,000; Gb, Gigabyte; bp, base pairs. [a]Per one SMRT cell. [b]Price ranges are based on a quotation obtained from manufacturers in May 2022. [c]Official sale of the instrument has been discontinued.

diet history method. Questionnaires should include questions about usual intake and eating patterns, as well as requesting a food record from participants to verify dietary data. Additionally, visual prompts can be used to increase the accuracy of portion size estimation[118]. To allow meaningful comparisons and statistical analyses, information on the use of medication (prescription and non-prescription) is best derived directly from the medical record of the donor and is ideally recorded according to the anatomical therapeutic chemical code[119,120]. Other biological specimens, such as blood and urine, can be collected in parallel with stool samples and further analysed for specific clinical, physico-chemical or metabolic parameters, depending on the disease of interest[113]. Finally, comparative gut microbiome studies should include faecal calprotectin and CRP assessments; both of these proteins are biomarkers of intestinal inflammation and have been linked to microbiota perturbations[117,121].

Following the practical organization of sample collection and storage, faecal samples are processed in typical metagenomic workflows for DNA extraction, library preparation and sequencing (Fig. 2). For large cohorts, batch effects that could potentially lead to interpretation bias should be avoided (for example, by randomizing samples across sampling time points and treatments)[122]. Depending on the aim of the study, available bioinformatics capacity and research budget, various approaches can be followed post-library preparation (Table 3). In addition to the continuous improvement of metagenomic workflows (Box 3) in terms of efficiency, quality control, automation and throughput, it is equally important to understand and control for factors that might greatly affect downstream analysis and thus the outcome of any metagenomic profiling study[123]. For instance, sampling of the human gut microbiome often results in uneven sampling depth (that is, the ratio of the number of sequenced cells to the actual number of cells present in samples), which can affect results in comparative analyses of unevenly distributed or diverse microbiomes. To correct for such events and thus limit correlation biases, relative microbial profiling should be complemented by quantitative microbial profiling, which also integrates bacterial cell counts obtained from flow cytometry[123,124]. In line with the increasing focus on quantitative approaches, the microbiome field is also gradually shifting from compositional to functional analyses based on whole-genome shotgun sequencing data. Here, the availability of well-curated microbial gene databases has a pivotal role. The largest and most widely used database used in this context is the Kyoto Encyclopedia of Genes and Genomes, which enables genome annotation and investigation of biological pathways[125]. The creation of gene catalogues provides a profound mechanistic understanding of the functional activities of the gut microbiota, how these functions can be influenced by environmental or host-specific factors and how they can affect the host functions. In addition, such insights facilitate complementary -omics approaches[126]. Whereas the value of genomic profile annotations is limited to the functional potential of the microbiome, more in-depth information about the activity of specific species can be acquired, for example, by determining protein abundances (meta proteomics) or metabolite concentrations (metabolomics)[127]. Microbial peptides, proteins and other metabolic products can be measured in faecal, blood, tissue and urine samples via both targeted and untargeted analyses[128]. In the specific case of CKD, the implementation of a multi-omics approach to obtain a systems biology-oriented view on gut microbiome functionality capturing intestinal production, absorption into the bloodstream and excretion via the urine of these products has been recommended[64].

Conclusions

Our understanding of the human gut microbiome has come a long way since the start of the Human Microbiome Project in 2007 (ref.[127]). This progress has been mainly driven by technological advances and a growing focus on larger and better defined cohorts, well-controlled study

Box 3

Major platforms for next-generation sequencing

- Illumina sequencing platforms revolutionized and dominated the field of sequencing by constantly improving its technologies such as the release of the NovaSeq 6000 instrument. Subsequently, other companies have also developed alternative third-generation sequencing technologies[169].
- Oxford Nanopore Technologies (ONT) offers longer read lengths than Illumina and has been increasingly used as a potential alternative (Table 3). The ONT technology is considered somewhat inferior to most Illumina instruments in terms of error rate in the particular cases of high-GC content and homopolymers accuracy[170].
- Pacific BioSciences (PacBio) sequencers focus on single molecule sequencing and offer an even longer read length sequencing than ONT, thus further improving the taxonomic identification of reads and allowing for detection of highly repetitive regions in microbial genomes[171,172]. Similar to ONT, however, the PacBio platform also has a higher error rate and lower throughput than Illumina, which may pose challenges to quantitative data analysis[169].
- A solution to the aforementioned issues may be the use of a hybrid Illumina/PacBio model, which can correct for errors in long reads and improve accuracy[170]. However, this approach might increase the sequencing cost and restrict its availability to a limited number of larger laboratories.
- Regardless of the platform used, one of the common future goals in the field is the assembly of metagenome-assembled genomes and creation of gene catalogues to explore the composition and functionality of microbes in the faecal sample[126].
- The lack of an error-free, widely accessible and broadly applicable sequencing instrument continues to present challenges in the gut microbiome field. Future availability of high-throughput platforms that are less error prone might address the limitations of the current technology.

designs and broad metadata collections. These improvements have led to novel insights into the compositional and functional nature of gut microbiome changes in an ever-growing number of human diseases and disorders, including intestinal dysbiosis in CKD and its link to the production of uraemic toxins as a risk factor for CVD. Although metagenomics has massively advanced our knowledge of host–microbiome relationships in health and disease, and will shape future approaches for assessing clinical diagnosis and therapeutic outcomes, the field needs to move beyond correlations and associations to reach clinical breakthroughs. This ambition calls for studies in which the multitude of complex interactions between community members and their host can be studied and manipulated in well-controlled models, which is a crucial prerequisite in the development of intervention strategies to prevent or decelerate the progression of CKD and its associated comorbidities[129]. In addition to the further technological refinement of in vitro fermentation systems[129] and microphysiological model systems[32], another key element in such interaction studies is the availability of well-documented isolates of bacterial species with specific roles in the intestinal metabolism of uraemic toxins or CKD-associated dysbiosis, as inferred from metagenomic data. Here, the renaissance of anaerobic culturing[130] and the vast number of innovative workflows for high-throughput single-cell isolation offer myriad opportunities for targeted and untargeted isolations of functionally important microbes from individuals with and without CKD[131]. The ultimate goal is to translate the ex vivo findings into personalized therapeutic strategies that can be easily developed and delivered to patients. Such therapies must be efficient in adapting or complementing the gut microbiota of the patient, while avoiding undesirable consequences. The low cost, minimal side effects and high tolerability of biotics suggest that they are promising add-on therapeutic options, although much remains to be elucidated regarding their efficacy in CKD. A silver bullet is not yet within reach, but the next generation of gut–kidney axis studies should be aimed at further improving study designs, method standardization and thus overall better inter-comparability between studies to accelerate improvements in the field.

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Competing interests
S.A.O., A-M.M., J.G. and J.v.B. are employees of Danone Nutricia Research. The other authors declare no competing interests.

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