Life Sciences 351 (2024) 122863

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Gut microbiota dynamics and association with chronic kidney disease: A longitudinal study within the PREDIMED-Plus trial

Alessandro Atzeni [a,b,c,*], Andrés Díaz-López [b,c,d], Adrián Hernández Cacho [a,b,c], Nancy Babio [a,b,c], Jesús F. García-Gavilán [a,b,c], Isabel Cornejo-Pareja [c,e], Clara Belzer [f], Montserrat Fitó [c,g], Francisco J. Tinahones [c,e], Jordi Salas-Salvadó [a,b,c,*]

[a] Alimentació, Nutrició, Desenvolupament i Salut Mental (ANUT-DSM), Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, Reus, Spain
[b] Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain
[c] Centro de Investigación Biomédica en Red - Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[d] Nutrition and Mental Health (NUTRISAM) Research Group, Universitat Rovira i Virgili, Reus, Spain
[e] Department of Endocrinology and Nutrition, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Málaga, Spain
[f] Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands
[g] Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d'Investigació Médica (IMIM), Barcelona, Spain

ARTICLE INFO

Keywords:
Gut microbiota
Chronic kidney disease
Cystatin C
eGFR

ABSTRACT

Aims: Chronic kidney disease (CKD) represents a global health concern, disproportionately affecting the elderly with heightened cardiovascular risk. The emerging focus on the gut microbiota's role in CKD pathophysiology represents a pivotal area in nephrology; however, the evidence on this topic is limited. This observational prospective study, in the framework of the PREDIMED-Plus trial, investigates associations between gut microbiota composition and the 1-year trajectory of CKD in 343 participants aged 55–75 years with high cardiovascular risk.

Materials and methods: Kidney function was assessed at baseline and at 1-year of follow-up through the estimated glomerular filtration rate based on cystatin C (eGFR-CysC) and CKD defined by eGFR-CysC <60 mL/min/1.73 m^2. Participants were grouped based on their 1-year CKD trajectory: Group 1 maintained normal status or improved from CKD to normal, while Group 2 maintained CKD or worsened from normal to CKD. Fecal microbiota composition was assessed through 16S sequencing.

Key findings: We observed differences in gut microbiota composition between CKD trajectory groups. Notably, the baseline relative abundance of *Lachnoclostridium* and *Lachnospira*, both butyrate-producing genera, was lower in participants maintaining or progressing to CKD. Longitudinally, a decrease in *Lachnospira* abundance was associated with CKD progression. The improved Chao1 index after 1-year follow-up suggests a link between enhanced microbial richness and stable/better kidney function.

Significance: The findings underscore the potential of gut microbiota analysis in non-invasively monitoring CKD, especially in older populations, and hint at future interventions targeting gut microbiota to manage CKD progression. Further research is needed for causal relationships and generalizability.

1. Introduction

Chronic kidney disease (CKD) is a global health concern, with recent data indicating a prevalence between 8 and 16 % [1], constantly increasing particularly among elderly individuals at high risk of cardiovascular complications [2]. This condition, characterized by a persistent abnormal kidney function (estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and albuminuria ≥30 mg/g), ranks as the 16th leading cause of reduced life years worldwide [1], and is associated with elevated morbidity and premature death, representing a substantial economic burden on healthcare. This underscores the global health impact of CKD, particularly in older populations, and highlights the need for a comprehensive understanding of the disease to implement suitable preventive measures.

In this regard, the potential causal role of gut microbiota alterations, also known as dysbiosis, in the pathophysiology of CKD represents a

* Corresponding authors at: Facultat de Medicina i Ciències de la Salut, Carrer Sant Llorenç, 21, 43201 Reus, Spain.
E-mail addresses: alessandro.atzeni@urv.cat (A. Atzeni), jordi.salas@urv.cat (J. Salas-Salvadó).

https://doi.org/10.1016/j.lfs.2024.122863
Received 8 May 2024; Received in revised form 19 June 2024; Accepted 19 June 2024
Available online 21 June 2024

prominent and innovative topic in nephrology literature [3]. Studies in rodent models showed that gut dysbiosis contributes to the development of kidney disease [4]. Furthermore, studies in humans also suggest that the gut microbiota alpha and beta-diversity may differ between populations with and without CKD. However, speculation arises about whether these differences contribute to the observed higher eGFR decline and disease progression risk in CKD patients, or if CKD itself leads to gut dysbiosis, given their bidirectional relationship [5,6].

Active research areas focus on understanding the consequences of gut microbiota composition and diversity variations on CKD development and progression. Moreover, these aspects may not be fully covered. In fact, some limitations of the available evidence need to be addressed: (i) most previous studies are cross-sectional rather than longitudinal studies; (ii) the vast majority of studies on this topic mainly measured kidney function (i.e., eGFR) using serum creatinine rather than Cystatin C (CysC), which is often considered a more accurate marker unaffected by sex, age, protein intake, and muscle mass [7]; (iii) earlier research focused on adults or middle-aged individuals with CKD, encompassing those at advanced CKD stages and end-stage kidney disease. However, it did not specifically include vulnerable elderly individuals with underlying comorbid conditions such as obesity/overweight and metabolic syndrome (MetS), where distinct profiles of gut microbiota have been previously documented [8]. Recently, Peters et al. also suggested that the relationship between kidney function, kidney damage, and the gut microbiome depends on diabetes status [9]. Finally, there have been few, if any, studies conducted in Mediterranean elderly populations, where individuals' socio-demographic and lifestyle traits (e.g., dietary habits, physical activity, smoking status, medication use, and so on) may protect against kidney function decline and in turn, reflect differences in their gut microbiome [10]. However, previous studies poorly accounted the influence of these relevant confounders when establishing the association between gut microbiota and CKD. Further research is needed to clarify the role of gut microbiota in CKD development and progression.

Therefore, the aim of the present observational study, in the framework of the PREDIMED-Plus (Prevención con Dieta Mediterránea-Plus) randomized trial, was to examine the baseline and longitudinal associations of gut microbiota composition and diversity with CKD progression, assessed through eGFR based on cystatin C (eGFR-CysC), in a large Spanish cohort of older individuals at high cardiovascular risk, within the context of a weight-loss lifestyle intervention.

2. Methods

2.1. Study design and participants

The present study, conducted within the framework of the PREDIMED-Plus randomized clinical trial using an observational prospective design, includes 343 participants from the Reus recruiting center. These participants had available fecal microbiota 16S data and information regarding eGFR-CysC at baseline and after 1-year of follow-up. The trial is described in detail in the Supplemental Material.

2.2. General assessments, anthropometric measurements, and blood biochemical parameters

Further details on this part of the methodology are provided in the Supplemental Material.

2.3. Kidney disease markers and outcome assessment

Further details on this part of the methodology are provided in the Supplemental Material.

The main outcome of interest for the present study was CysC-based CKD 1-year trajectory. Participants were considered to have CKD when eGFR-CysC was lower than 60 (mL/min/1.73). Study population was stratified in two groups according to CKD 1-year trajectory: participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2).

2.4. Stool samples collection, fecal bacterial DNA extraction, and 16S amplicon sequencing

Further details on this part of the methodology are provided in the Supplemental Material.

2.5. Statistical analyses

A detailed description of the statistical analyses conducted is provided in the Supplemental Material.

The clinical characteristics of the study population were described according to groups of CKD 1-year trajectory, and between-group differences were tested with Pearson's chi-squared test or Student's independent samples *t*-test. The overall microbiota structure (alpha diversity and beta diversity) and differentially abundant taxa (at the genus level) were the primary exposure of this study. The statistical analyses were adjusted for: the PREDIMED-Plus study group (intervention group, control group), age categories (below the median, ≤65 years old; above the median, >65 years old), sex, smoking status (former smoker, never smoker, smoker), previous diagnosis of hypertension, hypercholesterolemia, and type 2 diabetes, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2), MedDiet adherence score categories (high, 10 to 17; medium, 7 to 9; low, 2 to 6), and use of angiotensin II receptor antagonists (ARA II) and angiotensin-converting enzyme (ACE) drugs. The association between CKD 1-year trajectory groups and baseline and 1-year changes in calculated alpha diversity indexes Chao1, Shannon, and Simpson [11–13] was assessed through linear regression. Beta diversity was evaluated using Euclidean distance over log-ratio transformed genus counts (Aitchison distance) [14]. Permutational multivariate analysis of variance (PERMANOVA) tested differences in Aitchison distance between CKD 1-year trajectory groups cross-sectionally and longitudinally. Principal component analysis (PCA) was employed to visualize between-group and between-time variation. The differential abundance of fecal microbiota features across CKD 1-year trajectory groups was explored cross-sectionally and longitudinally using general linear models.

3. Results

3.1. General characteristics of the study population

The flow diagram in the Supplementary Fig. S1 indicates how the final sample size was obtained.

The baseline characteristics of the study population were described according to CKD 1-year trajectory groups (Table 1). From a total of 343 participants, 253 subjects were included in Group 1 (maintained normal or improved their status from CKD to normal after 1 year), and 90 subjects in Group 2 (maintained CKD or worsened their status from normal to CKD after 1 year). Participants in Group 1 were younger than participants in Group 2 (64.0 ± 4.8 versus 66.9 ± 5.1 years old). In addition, due to the kidney disease status, subjects in Group 2 had higher baseline levels of CysC (1.3 ± 0.2 mg/L), and lower eGFR-CysC (54.6 ± 10.5 mL/min/1.73 m^2) compared to subjects in Group 1 (1.0 ± 0.1 mg/L, and 79.0 ± 14.6 mL/min/1.73 m^2).

Follow up changes according to CKD 1-year trajectory groups are reported in Supplementary Table S1.

3.2. The baseline fecal microbiota and groups of CKD 1-year trajectory

We did not observe significant differences in calculated alpha

Table 1
Baseline characteristics of the study population according to chronic kidney disease (CKD) 1-year trajectory groups.

	Group 1 ($n = 253$)	Group 2 ($n = 90$)	Total ($n = 343$)	*P* value
Women	107 (42.3)	45 (50.0)	152 (44.3)	0.206
Age (years)	64.0 ± 4.8	66.9 ± 5.1	64.8 ± 5.1	< 0.001
Intervention group	135 (53.4)	43 (47.8)	178 (51.9)	0.363
Smoke Status				0.006
Former smoker	113 (44.7)	25 (27.8)	138 (40.2)	
Never smoker	116 (45.8)	48 (53.3)	164 (47.8)	
Smoker	24 (9.5)	17 (18.9)	41 (12.0)	
Hypercholesterolemia prevalence	165 (65.2)	61 (67.8)	226 (65.9)	0.660
Hypertension prevalence	199 (78.7)	79 (87.8)	278 (81.0)	0.058
Type 2 diabetes prevalence	40 (15.8)	22 (24.4)	62 (18.1)	0.068
Obesity (BMI ≥ 30 kg/m^2)	194 (76.7)	69 (76.7)	263 (76.7)	0.998
ARA II use	60 (23.7)	30 (33.3)	90 (26.2)	0.121
ACE inhibitors use	91 (36.0)	34 (37.8)	125 (36.4)	0.437
Adherence to MedDiet				0.921
High (10 to 15)	68 (26.9)	24 (26.7)	92 (26.8)	
Medium (7 to 9)	76 (30.0)	29 (32.2)	105 (30.6)	
Low (2 to 6)	109 (43.1)	37 (41.1)	146 (42.6)	
Physical activity (METs/day)	366.2 ± 340.2	360.7 ± 369.0	364.8 ± 347.5	0.896
HDL cholesterol (mg/dL)	48.7 ± 11.6	47.7 ± 11.6	48.4 ± 11.6	0.512
LDL cholesterol (mg/dL)	120.6 ± 28.9	118.3 ± 30.0	120.0 ± 29.1	0.527
TG (mg/dL)	173.3 ± 110.1	175.0 ± 87.6	173.8 ± 104.6	0.893
FPG (mg/dL)	118.2 ± 22.0	120.4 ± 26.0	118.8 ± 23.1	0.450
Insulin (mU/mL)	20.1 ± 10.5	20.2 ± 13.5	20.1 ± 11.4	0.941
Glycated hemoglobin (% over total)	6.0 ± 0.7	6.1 ± 0.9	6.0 ± 0.8	0.252
DBP (mmHg)	82.9 ± 8.6	81.2 ± 9.1	82.5 ± 8.7	0.103
Urinary creatinine (mg/dL)	104.6 ± 53.0	93.3 ± 43.8	101.7 ± 50.9	0.052
UACR (mg/g)	16.8 ± 46.2	21.9 ± 52.2	18.1 ± 47.8	0.403
CysC (mg/L)	1.0 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	< 0.001
eGFR-CysC (mL/min/1.73 m^2)	79.0 ± 14.6	54.6 ± 10.5	72.6 ± 17.3	< 0.001

BMI, body mass index; ARA II, angiotensin II receptor antagonists; ACE, angiotensin-converting enzyme; MedDiet, Mediterranean diet; METs, metabolic equivalents; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; FPG, fasting plasma glucose; DBP, diastolic blood pressure; UACR, urine albumin-to-creatinine ratio; CysC, cystatin C; eGFR-CysC, estimated glomerular filtration rate based on cystatin C. Groups of CKD 1-year trajectory defined including participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2). Data represented as number (% within CKD 1-year trajectory groups) or mean ± standard deviation. Differences between groups assessed by Pearson's chi-squared test or independent samples *t*-test. $P < 0.05$ deemed significant.

diversity indices Chao1, Simpson, and Shannon between groups of CKD 1-year trajectory (Supplementary Tables S2-S4). Principal components calculated over baseline taxonomic counts showed that the top 2 axes account for 11.1 % and 7.0 % of the total variation respectively (Supplementary Fig. S2). We observed significant variance ($P = 0.026$) in the fecal microbiota population, with groups of CKD 1-year trajectory explaining 0.5 % of all variance (Table 2).

The relative abundance of *Lachnoclostridium* genus was significantly lower (β coef. = −0.521, $P < 0.001$, FDR = 0.022) in Group 2 compared with Group 1 of CKD 1-year trajectory (Fig. 1A). Similarly, the relative abundance of *Lachnospira* genus was significantly lower (β coef. = −0.567, $P < 0.001$, FDR = 0.022) in Group 2 compared with Group 1 of CKD 1-year trajectory (Fig. 1B). On the other hand, we did not observe any differentially abundant pathway between groups of CKD 1-year trajectory.

3.3. Fecal microbiota changes and groups of CKD 1-year trajectory

The alpha diversity index Chao1 decreased (−0.5 ± 20.0) in those subjects belonging to Group 2 after 1 year (β coef. = −5.255, $P = 0.046$) (Fig. 2). No between-group significant differences were shown for Simpson and Shannon indices (Supplementary Tables S5-S6). We did not observe significant variance in gut microbiota population explained by groups of CKD 1-year trajectory assessed longitudinally through PERMANOVA (Supplementary Table S7). Principal components calculated over baseline and 1-year taxonomic counts showed that the top 2 axes account for 11.0 % and 6.8 % of the total variation respectively (Supplementary Fig. S3).

The relative abundance of *Lachnospira* genus was decreased (β coef. = −0.434, $P = 0.001$, FDR = 0.084) in Group 2 compared with Group 1 of CKD 1-year trajectory after 1 year (Fig. 3). No differences in pathways for the 1-year change were observed between groups of CKD 1-year trajectory.

4. Discussion

Our study explored both the cross-sectional and longitudinal

Table 2
Result of permutational multivariate analysis of variance (PERMANOVA) conducted to assess differences in Aitchison distance, calculated over baseline counts, between groups of chronic kidney disease (CKD) 1-year trajectory.

	Df	Sum of sqs	R2	F	*P* value
CKD 1-y trajectory group	1	158.373	0.005	1.602	0.018
PREDIMED-Plus study group	1	70.070	0.002	0.709	0.899
Smoke status	2	230.595	0.007	1.167	0.166
Type 2 diabetes	1	225.363	0.007	2.280	0.005
Sex	1	153.863	0.005	1.557	0.022
BMI category	1	94.744	0.003	0.959	0.512
Age category	1	134.084	0.004	1.357	0.088
MedDiet adherence category	2	209.937	0.006	1.062	0.300
Hypertension	1	77.634	0.002	0.786	0.793
Hypercholesterolemia	1	111.813	0.003	1.131	0.269
ARA II	1	97.659	0.003	0.988	0.445
ACE	1	92.311	0.003	0.934	0.555
Residual	327	32,318.367	0.947		
Total	342	34,120.154	1		

Df, degrees of freedom; BMI, body mass index; MedDiet, Mediterranean diet; CKD, chronic kidney disease; ARA II, angiotensin II receptor antagonists; ACE, angiotensin-converting enzyme. Groups of CKD 1-year trajectory defined including participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2). PERMANOVA was conducted to assess longitudinally differences between the interaction term group*time, setting participants' IDs as a variable within which to constrain permutations. Model adjusted for the PREDIMED-Plus study group (intervention group, control group), age categories (below the median, ≤ 65 years old; above the median, > 65 years old), sex, smoking status (former smoker, never smoker, smoker), prevalence of hypertension, hypercholesterolemia, and type 2 diabetes, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2), MedDiet adherence score categories (high, 10 to 17; medium, 7 to 9; low, 2 to 6), use of ARA II and ACE drugs. Results with $P < 0.05$ were considered significant.

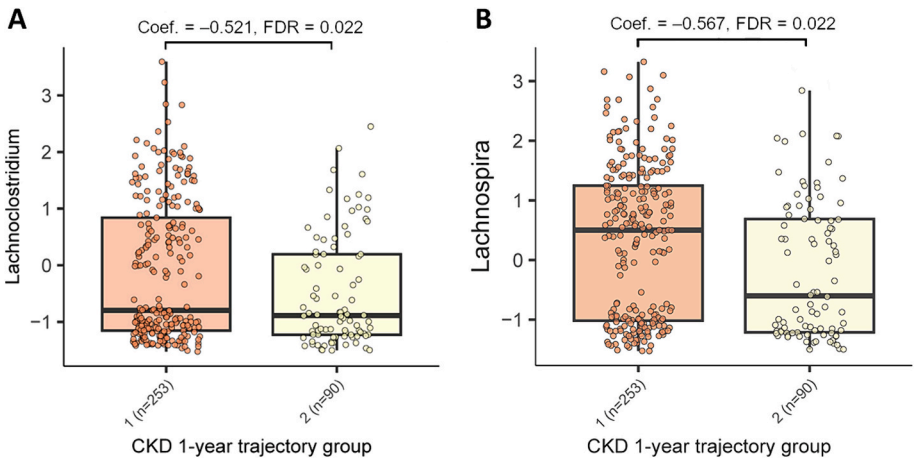

Fig. 1. Baseline differentially abundant taxa between groups of chronic kidney disease (CKD) 1-year trajectory. Groups of CKD 1-year trajectory defined including participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2). Multivariable association tested with generalized liner model, with Group 2 as reference, adjusted for the PREDIMED-Plus study group (intervention group, control group), age categories (below the median, ≤65 years old; above the median, >65 years old), sex, smoking status (former smoker, never smoker, smoker), prevalence of hypertension, hypercholesterolemia, and type 2 diabetes, body mass index (BMI) categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2), Mediterranean diet adherence score categories (high, 10 to 17; medium, 7 to 9; low, 2 to 6), use of angiotensin II receptor antagonists (ARA II), use of angiotensin-converting enzyme (ACE) drugs. Values in y axe indicate log-transformed relative abundance of genera with FDR < 0.1.

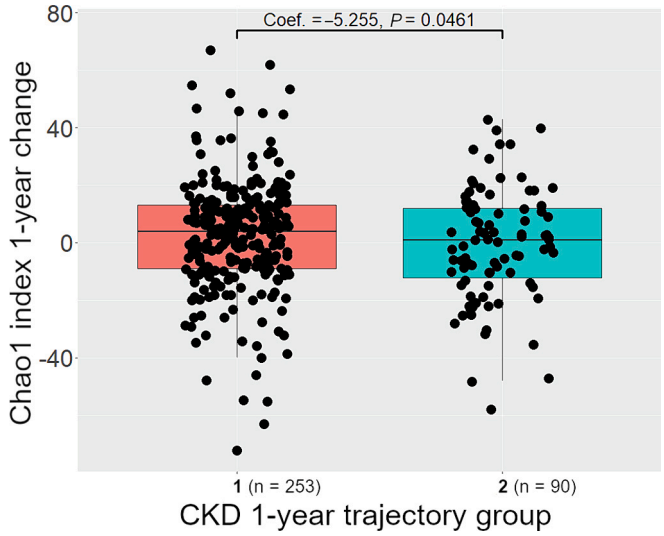

Fig. 2. Difference in Chao1 alpha diversity index 1-year change between chronic kidney disease (CKD) 1-year trajectory groups. Groups of CKD 1-year trajectory defined including participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2). Differences tested with linear regression, with Group 2 as reference, and model adjusted for the PREDIMED-Plus study group (intervention group, control group), age categories (below the median, ≤65 years old; above the median, >65 years old), sex, smoking status (former smoker, never smoker, smoker), prevalence of hypertension, hypercholesterolemia, and type 2 diabetes, body mass index (BMI) categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2), Mediterranean diet adherence score categories (high, 10 to 17; medium, 7 to 9; low, 2 to 6), use of angiotensin II receptor antagonists (ARA II), use of angiotensin-converting enzyme (ACE) drugs. Results with $P < 0.05$ were considered significant.

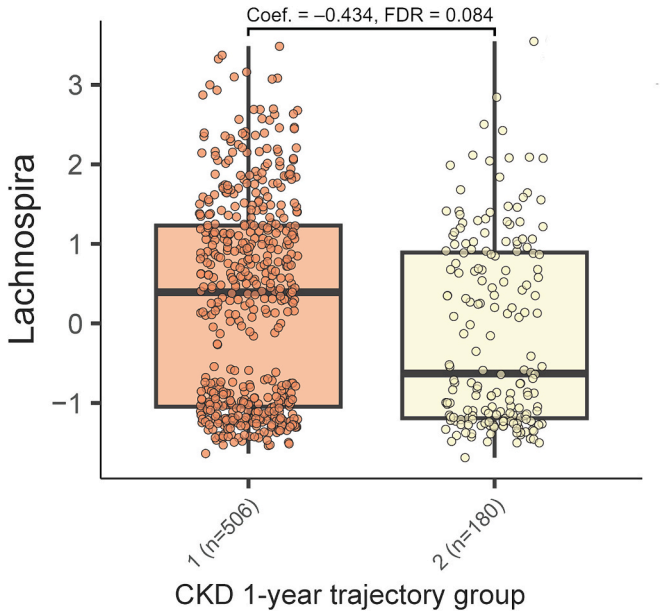

Fig. 3. Longitudinal multivariable association between differentially abundant taxa and groups of chronic kidney disease (CKD) 1-year trajectory. Groups of CKD 1-year trajectory defined including participants who maintained their status as normal or improved their status from CKD to normal after 1 year (Group 1), or participants who maintained CKD or worsened their status from normal to CKD after 1 year (Group 2). Association tested with generalized liner model, with Group 2 as reference, adjusted for the time between baseline and follow-up, the PREDIMED-Plus study group (intervention group, control group), age categories (below the median, ≤65 years old; above the median, >65 years old), sex, smoking status (former smoker, never smoker, smoker), prevalence of hypertension, hypercholesterolemia, and type 2 diabetes, body mass index (BMI) categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2), Mediterranean diet adherence score categories (high, 10 to 17; medium, 7 to 9; low, 2 to 6), use of angiotensin II receptor antagonists (ARA II), use of angiotensin-converting enzyme (ACE) drugs. Participants' ID was specified as random effect. Values in y axe indicate log-transformed relative abundance of genera with FDR < 0.1.

relationships between CKD, classified according to eGFR-CysC, and gut microbiota in elderly individuals at high cardiovascular risk, within the context of a weight-loss lifestyle intervention. We observed differences in the gut microbiota between groups of CKD 1-year trajectory. Individuals who either maintained CKD or progressed from normal to CKD

status, exhibited a lower relative abundance of butyrate-producing genera from Lachnospiraceae family, *Lachnoclostridium* and *Lachnospira* at baseline. Furthermore, we observed a decrease in gut microbiota richness and in the relative abundance of genus *Lachnospira* in individuals who either maintained CKD or progressed from normal to CKD status after one year.

Similarly, differences in the overall gut microbial composition, including alterations in Lachnospiraceae abundance, have been observed between controls and patients with CKD [5]. A systematic review proposed by Zhao and colleagues aimed to profile the gut microbiota in patients with CKD [15]. The 67 % of the studies considered, reported lower gut microbiota richness, whereas the 91 % reported distinct bacterial composition in patients compared to healthy controls [15]. In addition, Lachnospiraceae were described less abundant in CKD patients [15].

Members of the Lachnospiraceae family, anaerobic organisms in the Clostridium order, convert plant-based polysaccharides into short-chain fatty acids like butyrate [16]. Butyrate plays a crucial role in overall health, by preserving the intestinal epithelium, influencing the immune system, and affecting different metabolic pathways in various organs [17]. A reduction in butyrate-producing bacteria in the gut has been associated with several noncommunicable diseases like type 2 diabetes, obesity, and cardiovascular diseases [18]. Additionally, a decrease in these bacteria can promote the growth of harmful gut pathogens by weakening the body's natural resistance to colonization [18]. In the specific context of CKD, *Lachnospira* has been already identified as potential biomarker [19]. Accordingly, a meta-analysis focusing on gut microbiota alterations in membranous nephropathy showed that the abundance of *Lachnospira* was markedly depleted in patients compared to healthy controls [20]. Furthermore, higher *Lachnospira* abundance has been observed in control groups compared to patients with CKD. Gut microbiota alterations become more pronounced from CKD stage 3b (eGFR between 30 and 44 mL/min per 1.73 m^2) onwards, and persists even after renal replacement therapy [16].

Together with *Lachnospira*, we also observed lower abundance of *Lachnoclostridium* in subjects who either maintained CKD or progressed from normal to CKD status. Regarding *Lachnoclostridium*, literature show more controversial results, as it has been found depleted [21,22], but also enriched [23,24] in patients with CKD. The ability of *Lachnoclostridium* to produce butyrate from dietary fibers could in part explain its potential beneficial impact on kidney function. On the other hand, *Lachnoclostridium* it has been identified as microbial trimethylamine (TMA) producing genera [25]. The association between TMA N-oxide (TMAO) and kidney disease is supported by a study in animal models showing elevated TMAO levels, which are linked to reduced kidney function and increased mortality risk, partially explaining the observed literature discrepancy [26].

Interestingly, while baseline comparisons showed a variance in microbiota between the two CKD trajectory groups, the longitudinal analysis revealed a decrease in *Lachnospira* abundance in the group with worsening CKD. This suggests a potential role of *Lachnospira* in the progression of CKD, especially in the context of dietary interventions. It also indicates that changes in gut microbiota may not just be a consequence of CKD but could actively contribute to its progression. As emphasized by Wehedy et al., there is a two-way interaction between CKD and the gut microbiome [27]. Changes in gut microbiota can impact kidney health by promoting inflammation and accumulating uremic toxins, while CKD can lead to an imbalance in gut bacteria [27].

The observed differences in the gut microbial composition between groups of CKD 1-year trajectory suggest that changes in the microbiota are associated with the progression or improvement of CKD. This reinforces the concept that gut microbiome composition is crucial CKD dynamics, with alterations potentially contributing to CKD progression or being influenced by changes in kidney function [28,29].

The observed 1-year increase in the Chao1 alpha diversity index in individuals of Group 1 suggests a possible link between improved gut microbial richness and stable or better kidney function. This supports the idea that a diverse gut microbiome benefits overall and renal health [30], contributing to evidence that gut microbiota diversity may play a role in the progression and management of CKD [30].

The absence of significant changes in other diversity indices and the lack of differential abundant metabolic pathways over one year suggest a complex interplay between gut microbiota composition and CKD, which is not solely explained by changes in microbial diversity or functional potential. This complexity underscores the need for further research to unravel the mechanisms behind these associations and their implications for CKD management.

Our study adds to the evidence linking gut microbiota to CKD and its progression, suggesting that monitoring and modulating gut microbiota could be crucial managing CKD, particularly in older populations. This aligns with previous research indicating a link between gut dysbiosis and kidney disease [31]. Our findings also highlight the potential of gut microbiome analysis as a non-invasive tool for monitoring CKD progression, especially when traditional biomarkers like eGFR based on serum creatinine are less reliable [32].

A study by Jiang et al., explored the relationship between eGFR-CysC and gut microbiota in patients with End-Stage Renal Disease (ESRD), finding that levels of Cystatin C, along with markers like BUN and creatinine, were significantly higher in ESRD patients compared to healthy controls, and eGFR was reduced. The study also analyzed the diversity and distribution of gut microbiota in these patients, demonstrating a significant relationship between kidney function and gut microbiota composition [33]. Our study's reliance on eGFR-CysC for evaluating kidney function sets our research apart, as eGFR-CysC is considered more accurate in certain populations, especially the elderly, and less influenced by external factors like muscle mass compared to creatinine-based measures [34]. This approach adds robustness to our findings about the relationship between CKD progression and gut microbiota changes, underscoring the relevance of using eGFR-CysC in CKD studies, particularly in older adults, for more precise assessments of kidney function and its association with gut health.

Strengths of our study include the use of eGFR-CysC for kidney function assessment, which offers greater accuracy in elderly populations. The longitudinal design and integration of microbiome analysis add depth to our understanding of CKD progression.

However, an important limitation of this study is the lack of causal relationship establishment between microbiota changes and CKD progression. The observational nature of the study design aimed to identify associations but limits the ability to infer causality. Although significant associations between gut microbiota composition and CKD trajectory were found in our study, we acknowledge the absence of a causal relationship between gut microbiota changes and CKD progression. Nonetheless, our findings offer valuable insights, which can help the development of future studies, that should employ experimental designs to manipulate gut microbiota composition and measure the effects on CKD progression.

To address this limitation, future experiments could employ randomized controlled trials to restore levels of *Lachnoclostridium* and *Lachnospira* and evaluate their effects on CKD progression. Specifically, participants with CKD could be randomly assigned to receive probiotics supplementations weather a control group would receive a placebo. By monitoring changes in gut microbiota composition and CKD biomarkers over time, researchers could assess weather restoring specific microbiota levels can causally impact CKD progression. This approach would allow to establish a causal relationship, providing more robust evidence for the potential of gut microbiota analysis in non-invasively monitoring CKD.

Further, animal models of CKD could be used to investigate causality. Germ-free or antibiotic-treated rodents could be colonized with microbiota from CKD patients or specific strains like *Lachnoclostridium* and *Lachnospira*. By observing the effects on kidney function and pathology, researchers could infer causality and explore underlying mechanisms.

Other limitations include the limited generalizability of the findings

to other populations and the use of 16S rRNA sequencing, which provides taxonomic information at the genus level but lacks functional insights. Future studies should include more diverse study populations and integrate metagenomic and metabolomic analyses alongside taxonomic profiling to understand the functional impact of microbiota on CKD.

In conclusion, our study highlights the complex relationship between CKD and gut microbiota, particularly in elderly individuals at high cardiovascular risk. The use of cystatin C-based eGFR as a more accurate measure of renal function, along with the analysis of microbiome changes over one year, provides valuable insights into the gut-kidney axis. Our findings suggest potential associations between specific gut bacteria, such as the genera *Lachnoclostridium* and *Lachnospira*, and CKD progression. Future possibilities stemming from our study include exploring interventions targeting gut microbiota to manage or slow down CKD progression. Investigating the causal relationships between specific microbiota changes and CKD in diverse populations could provide new insights. Further research into the gut-kidney axis might lead to novel diagnostic tools or therapeutic strategies, especially focusing on the role of cystatin C and gut microbiome diversity in CKD. Potential future interventions could involve modulating the gut microbiota to reduce uremic toxin production, decrease inflammation, and improve kidney function. Additionally, fecal microbiota transplantation from healthy individuals to those with CKD warrants future exploration, as it may help in to restore a balanced gut microbiota composition. These insights open avenues for future research focused on gut microbiota as both a therapeutic target and diagnostic tool in CKD management..

Funding

This work was supported by the official Spanish Institutions for funding scientific biomedical research, CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund: three coordinated FIS projects led by JS-S; the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to JS-S; the Recercaixa (number 2013ACUP00194) grant to JS-S; the Boosting young talent call grant program for the development of IISPV research projects 2019–2021 (Ref.: 2019/IISPV/03 grant to AD-L); the Societat Catalana d'Endocrinologia i Nutrició (SCEN) Clinical-Research Grant 2019 (IPs: JS-S and AD-L). Collaborative Nutrition and/or Obesity Project for Young Researchers 2019 supported by CIBEROBN entitled: Lifestyle Interventions and Chronic Kidney Disease: Inflammation, Oxidative Stress and Metabolomic Profile (LIKIDI study) grant to AD-L. This research was also partially funded by the Eat2beNICE/H2020-SFS-2016-2 EU-H2020 European grant. AD-L received a Serra Hunter Fellowship from Generalitat de Catalunya. JS-S, a senior author of this paper, was partially supported by ICREA under the ICREA Academia program. None of the funding sources took part in the design, collection, analysis, interpretation of the data, writing the report, or in the decision to submit the manuscript for publication.

CRediT authorship contribution statement

Alessandro Atzeni: Writing – original draft, Formal analysis. **Andrés Díaz-López:** Writing – review & editing. **Adrián Hernández Cacho:** Visualization. **Nancy Babio:** Visualization. **Jesús F. García-Gavilán:** Visualization. **Isabel Cornejo-Pareja:** Visualization. **Clara Belzer:** Visualization. **Montserrat Fitó:** Visualization. **Francisco J. Tinahones:** Visualization. **Jordi Salas-Salvadó:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declared no competing interests.

Data availability

The dataset generated and analyzed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scommittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

Acknowledgment

Authors thank all PREDIMED-Plus participants and investigators. CIBEROBN is an initiative of the Instituto de Salud Carlos III (ISCIII), Madrid, Spain. The Hojiblanca (Lucena, Spain) and Patrimonio Comunal Olivarero (Madrid, Spain) food companies donated extra-virgin olive oil. The Almond Board of California (Modesto, CA), American Pistachio Growers (Fresno, CA), and Paramount Farms (Wonderful Company, LLC, Los Angeles, CA) donated nuts for the PREDIMED-Plus pilot study. The authors also thank the PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples. JS-S senior author of this study gratefully acknowledges the financial support by ICREA under the ICREA Academia programme. The research groups want to thank Jasper Koehorst and Bart Nijsse from the Unlock platform for their support in processing the data with the NG-tax pipeline. We also want to thank Athanasia Ioannou for her work with the stool sample preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2024.122863.

References

[1] National Kidney Foundation. (n.d.), Estimated Glomerular Filtration Rate (eGFR), (n.d.). https://www.kidney.org/atoz/content/gfr (accessed December 12, 2023).
[2] National Kidney Foundation. (n.d.), Global Facts: About Kidney Disease, (n.d.). https://www.kidney.org/kidneydisease/global-facts-about-kidney-disease (accessed December 12, 2023).
[3] S. Amini Khiabani, M. Asgharzadeh, H. Samadi Kafil, Chronic kidney disease and gut microbiota, Heliyon 9 (2023) e18991, https://doi.org/10.1016/j.heliyon.2023.e18991.
[4] D.W. Randall, J. Kieswich, L. Hoyles, K. McCafferty, M. Curtis, M.M. Yaqoob, Gut dysbiosis in experimental kidney disease: a meta-analysis of rodent repository data, J. Am. Soc. Nephrol. 34 (2023) 533–553, https://doi.org/10.1681/ASN.0000000000000071.
[5] J. Stanford, K. Charlton, A. Stefoska-Needham, R. Ibrahim, K. Lambert, The gut microbiota profile of adults with kidney disease and kidney stones: a systematic review of the literature, BMC Nephrol. 21 (2020), https://doi.org/10.1186/s12882-020-01805-w.
[6] S. Al Khodor, I.F. Shatat, Gut microbiome and kidney disease: a bidirectional relationship, Pediatr. Nephrol. 32 (2017) 921–931, https://doi.org/10.1007/s00467-016-3392-7.
[7] J.S. Lees, E. Rutherford, K.I. Stevens, D.C. Chen, R. Scherzer, M.M. Estrella, M. K. Sullivan, N. Ebert, P.B. Mark, M.G. Shlipak, Assessment of cystatin C level for risk stratification in adults with chronic kidney disease, JAMA Netw. Open 5 (2022) e2238300, https://doi.org/10.1001/jamanetworkopen.2022.38300.
[8] M. Pinart, A. Dötsch, K. Schlicht, M. Laudes, J. Bouwman, S.K. Forslund, T. Pischon, K. Nimptsch, Gut microbiome composition in obese and non-obese persons: a systematic review and meta-analysis, Nutrients 14 (2021) 12, https://doi.org/10.3390/nu14010012.
[9] B.A. Peters, Q. Qi, M. Usyk, M.L. Daviglus, J. Cai, N. Franceschini, J.P. Lash, M. D. Gellman, B. Yu, E. Boerwinkle, R. Knight, R.D. Burk, R.C. Kaplan, Association of the gut microbiome with kidney function and damage in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), Gut Microbes 15 (2023), https://doi.org/10.1080/19490976.2023.2186685.
[10] L. Voroneanu, A. Burlacu, C. Brinza, A. Covic, G.G. Balan, I. Nistor, C. Popa, S. Hogas, A. Covic, Gut microbiota in chronic kidney disease: from composition to modulation towards better outcomes—a systematic review, J. Clin. Med. 12 (2023) 1948, https://doi.org/10.3390/jcm12051948.
[11] A. Chao, Estimating the population size for capture-recapture data with unequal catchability, Biometrics 43 (1987) 783, https://doi.org/10.2307/2531532.

[12] C.E. Shannon, A mathematical theory of communication, Bell Syst. Tech. J. 27 (1948) 623–656, https://doi.org/10.1002/j.1538-7305.1948.tb00917.x.
[13] E.H. Simpson, Measurment of diversity, Nature 163 (1949) 688, https://doi.org/10.1038/163688a0.
[14] J. Aitchison, C. Barceló-Vidal, J.A. Martín-Fernández, V. Pawlowsky-Glahn, Logratio analysis and compositional distance, Math. Geol. 32 (2000) 271–275, https://doi.org/10.1023/A:1007529726302.
[15] J. Zhao, X. Ning, B. Liu, R. Dong, M. Bai, S. Sun, Specific alterations in gut microbiota in patients with chronic kidney disease: an updated systematic review, Ren. Fail. 43 (2021) 102–112, https://doi.org/10.1080/0886022X.2020.1864404.
[16] T. Yasuno, K. Takahashi, K. Tada, H. Hiyamuta, M. Watanabe, K. Ito, H. Arima, K. Masutani, Dysbiosis of gut microbiota in patients with chronic kidney disease, Intern. Med. (2023), https://doi.org/10.2169/internalmedicine.1602-23.
[17] P.M. Smith, M.R. Howitt, N. Panikov, M. Michaud, C.A. Gallini, M. Bohlooly-Y, J. N. Glickman, W.S. Garrett, The microbial metabolites, short-chain fatty acids, regulate colonic T reg cell homeostasis, Science 341 (2013) (1979) 569–573, https://doi.org/10.1126/science.1241165.
[18] M. Vital, A. Karch, D.H. Pieper, Colonic butyrate-producing communities in humans: an overview using omics data, MSystems 2 (2017) 1–18, https://doi.org/10.1128/mSystems.00130-17.
[19] H. Lun, W. Yang, S. Zhao, M. Jiang, M. Xu, F. Liu, Y. Wang, Altered gut microbiota and microbial biomarkers associated with chronic kidney disease, Microbiologyopen 8 (2019) 1–10, https://doi.org/10.1002/mbo3.678.
[20] Y. Zhang, J. Zhao, Y. Qin, Y. Wang, Z. Yu, X. Ning, S. Sun, Specific alterations of gut microbiota in patients with membranous nephropathy: a systematic review and meta-analysis, Front. Physiol. 13 (2022) 1–12, https://doi.org/10.3389/fphys.2022.909491.
[21] H. Chen, J. Wang, Q. Ouyang, X. Peng, Z. Yu, J. Wang, J. Huang, Alterations of gut microbes and their correlation with clinical features in middle and end-stages chronic kidney disease, Front. Cell. Infect. Microbiol. 13 (2023) 1–11, https://doi.org/10.3389/fcimb.2023.1105366.
[22] F. Pivari, A. Mingione, G. Piazzini, C. Ceccarani, E. Ottaviano, C. Brasacchio, M. Dei Cas, M. Vischi, M.G. Cozzolino, P. Fogagnolo, A. Riva, G. Petrangolini, L. Barrea, L. Di Renzo, E. Borghi, P. Signorelli, R. Paroni, L. Soldati, Curcumin supplementation (Meriva®) modulates inflammation, lipid peroxidation and gut microbiota composition in chronic kidney disease, Nutrients 14 (2022), https://doi.org/10.3390/nu14010231.
[23] Z.X. Zhong, J.X. Tan, L. Tan, Y. Tang, Z.C. Qiu, G.Q. Pei, W. Qin, Modifications of gut microbiota are associated with the severity of IgA nephropathy in the Chinese population, Int. Immunopharmacol. 89 (2020) 107085, https://doi.org/10.1016/j.intimp.2020.107085.
[24] I.W. Wu, C.Y. Lin, L.C. Chang, C.C. Lee, C.Y. Chiu, H.J. Hsu, C.Y. Sun, Y.C. Chen, Y. L. Kuo, C.W. Yang, S.S. Gao, W.P. Hsieh, W.H. Chung, H.C. Lai, S.C. Su, Gut microbiota as diagnostic tools for mirroring disease progression and circulating nephrotoxin levels in chronic kidney disease: discovery and validation study, Int. J. Biol. Sci. 16 (2020) 420–434, https://doi.org/10.7150/ijbs.37421.
[25] Y.Y. Cai, F.Q. Huang, X. Lao, Y. Lu, X. Gao, R.N. Alolga, K. Yin, X. Zhou, Y. Wang, B. Liu, J. Shang, L.W. Qi, J. Li, Integrated metagenomics identifies a crucial role for trimethylamine-producing Lachnoclostridium in promoting atherosclerosis, NPJ Biofilms Microbiomes 8 (2022), https://doi.org/10.1038/s41522-022-00273-4.
[26] Q. Fang, B. Zheng, N. Liu, J. Liu, W. Liu, X. Huang, X. Zeng, L. Chen, Z. Li, D. Ouyang, Trimethylamine N-oxide exacerbates renal inflammation and fibrosis in rats with diabetic kidney disease, Front. Physiol. 12 (2021), https://doi.org/10.3389/fphys.2021.682482.
[27] E. Wehedy, I.F. Shatat, S. Al Khodor, The human microbiome in chronic kidney disease: a double-edged sword, Front Med (Lausanne) 8 (2022) 13–15, https://doi.org/10.3389/fmed.2021.790783.
[28] N.L. Rukavina Mikusic, N.M. Kouyoumdzian, M.R. Choi, Gut microbiota and chronic kidney disease: evidences and mechanisms that mediate a new communication in the gastrointestinal-renal axis, Pflugers Arch. 472 (2020) 303–320, https://doi.org/10.1007/s00424-020-02352-x.
[29] H. Krukowski, S. Valkenburg, A.-M. Madella, J. Garssen, J. van Bergenhenegouwen, S.A. Overbeek, G.R.B. Huys, J. Raes, G. Glorieux, Gut microbiome studies in CKD: opportunities, pitfalls and therapeutic potential, Nat. Rev. Nephrol. 19 (2023) 87–101, https://doi.org/10.1038/s41581-022-00647-z.
[30] M. Snelson, A. Biruete, C. McFarlane, K. Campbell, A renal clinician's guide to the gut microbiota, J. Ren. Nutr. 30 (2020) 384–395, https://doi.org/10.1053/j.jrn.2019.11.002.
[31] N. Zaidan, L. Nazzal, The microbiome and uremic solutes, Toxins (Basel) 14 (2022) 245, https://doi.org/10.3390/toxins14040245.
[32] N. Ebert, S. Bevc, A. Bökenkamp, F. Gaillard, M. Hornum, K.J. Jager, C. Mariat, B. O. Eriksen, R. Palsson, A.D. Rule, M. van Londen, C. White, E. Schaeffner, Assessment of kidney function: clinical indications for measured GFR, Clin. Kidney J. 14 (2021) 1861–1870, https://doi.org/10.1093/ckj/sfab042.
[33] S. Jiang, S. Xie, D. Lv, P. Wang, H. He, T. Zhang, Y. Zhou, Q. Lin, H. Zhou, J. Jiang, J. Nie, F. Hou, Y. Chen, Alteration of the gut microbiota in Chinese population with chronic kidney disease, Sci. Rep. 7 (2017) 2870, https://doi.org/10.1038/s41598-017-02989-2.
[34] A.S. Levey, J. Coresh, H. Tighiouart, T. Greene, L.A. Inker, Measured and estimated glomerular filtration rate: current status and future directions, Nat. Rev. Nephrol. 16 (2020) 51–64, https://doi.org/10.1038/s41581-019-0191-y.