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Lactobacillus acidophilus KBL409 improves serum indoxyl sulfate via gut microbial changes in a human study

Sung Jae Jang[1,2], SungJun Park[2,3,4] ✉, Kiuk Lee[3], Woon-Ki Kim[1,5], Sung Hyun Moon[3], Bo-Ram Cho[3], Cheonghoon Lee[1,5], Hyungjin Lee[2,3], Tae-Wook Nam[3,6] & GwangPyo Ko[1,2,3,4,5] ✉

Chronic kidney disease (CKD) is characterized by progressive attenuation of kidney functions, resulting in the accumulation of uremic toxins. Various symptoms for CKD are closely associated with the gut-kidney axis, which represents the interaction between gut microbiota and CKD. In this study, we investigated the effects of *Lactobacillus acidophilus* (*L. acidophilus*) KBL409 on uremic toxin concentrations using a multi-center, randomized, double-blind, placebo-controlled study. Participants in the *L. acidophilus* KBL409 group received a daily capsule containing 1×10^{10} colony-forming units of *L. acidophilus* KBL409 or placebo. The per protocol analysis included 34 participants in the *L. acidophilus* KBL409 group and 30 participants in the placebo group. After 16 weeks, the serum indoxyl sulfate (IS) concentration was significantly lower in the *L. acidophilus* KBL409 group than in the placebo group ($p < 0.05$). Additionally, significant reductions in the genera *Blautia*, *Butyricicoccus, Lachnospiraceae* UCG-004, and *Megamonas* were observed in the *L. acidophilus* KBL409 group. These bacteria exhibited positive correlations with predicted functional genes linked to uremic toxin synthesis pathways, suggesting that *L. acidophilus* KBL409 reduced serum IS by altering gut microbial compositions. Therefore, *L. acidophilus* KBL409 could be used as an effective probiotic for improving kidney health through gut microbiota modulation.

Chronic kidney disease (CKD) is a global public health concern characterized by progressive deterioration of kidney functions[1–3]. The gradual decline in kidney function due to CKD leads to the accumulation of blood urea and uremic toxins, including indoxyl sulfate (IS), *p*-Cresyl sulfate (PCS), and trimethylamine N-oxide (TMAO), eventually resulting in uremia[4–8]. Serum IS concentrations are strongly correlated with estimated glomerular filtration rate (eGFR) and have been associated with the progression of CKD and cardiovascular disease (CVD)[8,9].

Gut microbiota play a critical role in the complex network of human organs through microbial metabolites[10,11]. A dramatic shift in gut microbial composition can increase both the accumulation of uremic toxins in the blood and the risk of CKD[12,13]. The gut-kidney axis represents the interaction between CKD and gut microbiota or intestinal physiology, such as intestinal permeability[14,15]. IS, one of the major uremic toxins, is originated from the gut microbiota mediated metabolism of tryptophan, an essential amino acid derived from dietary protein[16]. Specific gut bacteria, including the genera *Alistipes*, *Bacteroides*, *Blautia*, *Butyricicoccus*, *Escherichia*, and *Ruminococcus*, have been reported to express tryptophanase, which can metabolize tryptophan into indole, the precursor of IS[17–19]. IS acts as an aryl hydrocarbon receptor agonist, inducing pro-inflammatory and pro-fibrotic effects through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) or mitogen-activated protein kinase (MAPK) signaling pathways[20–22].

Despite the lack of effective ways for controlling increased uremic toxin levels in the blood, recent studies have demonstrated significant reductions in uremic toxins, including IS and PCS, through alterations in gut microbiota using probiotics or prebiotics[23–25]. Notably, the genera *Bifidobacterium* and *Lactobacillus*, which are well-known probiotic microorganisms, exhibit differing abilities to reduce IS and PCS[26,27]. These differences highlight the need to investigate specific probiotic strains capable of effectively reducing uremic toxins for potential applications in CKD prevention.

[1]Department of Environmental Health Sciences, Graduate School of Public Health, Seoul National University, Seoul, Republic of Korea. [2]weBiom Inc., Seoul, Republic of Korea. [3]KoBioLabs, Inc, Seoul, Republic of Korea. [4]N-Bio, Seoul National University, Seoul, Republic of Korea. [5]Institute of Health and Environment, Seoul National University, Seoul, Republic of Korea. [6]Present address: MightyBugs Inc., Busan, Republic of Korea. ✉e-mail: haha7007@snu.ac.kr; gko@snu.ac.kr

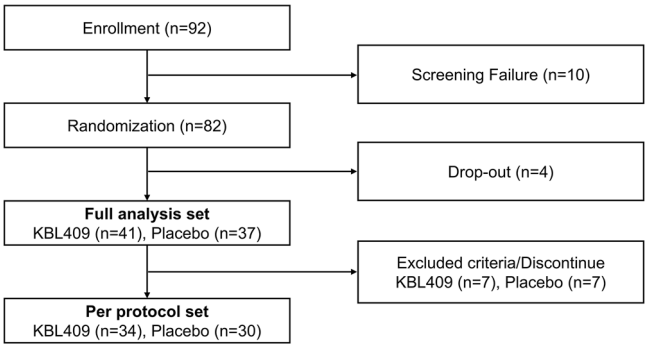

Fig. 1 | Flow diagram of the participant screening process.

Previously, we confirmed that *Lactobacillus acidophilus* (*L. acidophilus*) KBL409 has the potential to reduce serum levels of several uremic toxins, including IS and PCS, improve kidney function, attenuate inflammation and fibrosis, and enhance mitochondrial function in an adenine-induced CKD mouse model[28–30]. Therefore, in this study, we investigated the effects of *L. acidophilus* KBL409 on the improvement of serum IS concentrations through changes in gut microbial composition using a multicenter, randomized, double-blind, placebo-controlled study.

Results

Demographic characteristics

The per protocol set consisted of 34 participants in the *L. acidophilus* KBL409 group and 30 participants in the placebo group (Fig. 1). After randomization, 4 participants in the placebo group dropped out and 7 participants from each group were excluded from further analyses or withdrew from this study due to the reasons including the withdrawal of consent, the compliance score lower than 80%, the use of systemic antibiotics or the angiotensin II receptor blockers, the loss of capsules, and the adverse event. The mean ages of participants in the *L. acidophilus* KBL409 group and the placebo group were 55.4 ± 12.7 years and 56.4 ± 13.9 years, respectively (Table 1). The mean body mass index of the *L. acidophilus* KBL409 group was 26.4 ± 4.6 kg/m^2, which did not show a significant difference from the placebo group (25.7 ± 3.6 kg/m^2, $p = 0.5260$). The major lifestyle factors of both groups, including smoking, alcohol consumption, and exercise, showed no significant difference between the two groups.

Table 1 | Demographic characteristics

Variables[a]	*L. acidophilus* KBL409 group ($n = 34$)	Placebo group ($n = 30$)	*P*-value
Age (years)	55.4 ± 12.7	56.4 ± 13.9	0.7667[b]
Sex (M/F) (n, %)	22 (64.7)/12 (35.3)	20 (66.7)/10 (33.3)	0.8691[c]
Body mass index (kg/m^2)	26.4 ± 4.6	25.7 ± 3.6	0.5260[b]
Systolic blood pressure (mmHg)	125.0 ± 11.0	126 ± 12.6	0.7642[b]
Diastolic blood pressure (mmHg)	74.9 ± 8.8	76.8 ± 8.8	0.4118[b]
Smoking (n, %)			0.6311[c]
None	20 (58.8)	15 (50.0)	
Past	10 (29.4)	9 (30.0)	
Current	4 (11.8)	6 (20.0)	
Alcohol use per week (n, %)			0.6197[d]
None	20 (58.8)	13 (43.3)	
Past	5 (14.7)	4 (13.3)	
1 or less	8 (23.5)	10 (33.3)	
1–3	1 (2.9)	2 (6.7)	
≥3 or more	0 (0)	1 (3.3)	
Exercise per week (n, %)			0.1874[d]
None	9 (26.5)	7 (23.3)	
1–2	10 (29.4)	5 (16.7)	
3–4	8 (23.5)	9 (30.0)	
5–6	1 (2.9)	6 (20.0)	
Everyday	6 (17.6)	3 (10.0)	

[a]Data are presented as means ± SD or number.
[b]Inter-group comparisons were performed using a two-sample t-test.
[c]Inter-group comparisons were performed using the chi-square test.
[d]Inter-group comparisons were performed using Fisher's exact test.

Dietary patterns

The *L. acidophilus* KBL409 group showed a significant increase in the mean protein intake in Visit 5 (16 weeks; mean change: 10.38 ± 25.10 g, $p = 0.0237$) (Table S1). However, participants the placebo group did not show the significant increases in the mean protein intake in Visit 5 (mean change: 3.64 ± 14.69 g). There were no significant differences in the mean dietary fiber intake of both groups in Visit 5 (Table S1). The *L. acidophilus* KBL409 group also showed significantly increases in the mean intake of lipids (mean change: 9.21 ± 23.68 g, $p = 0.0326$), moisture (mean change: 110.96 ± 233.02 g, $p = 0.0101$), and crude ash (mean change: 2.37 ± 6.29 g, $p = 0.0378$) in Visit 5 (Table S1).

Effects of *L. acidophilus* KBL409 on kidney function indicators and uremic toxin concentrations

After 16 weeks of *L. acidophilus* KBL409 administration, decreases in kidney function indicators, including urine protein (mean change: −28.42 ± 170.81 mg/dL), urine creatinine (mean change: −4.19 ± 82.55 mg/dL), and UPCR (mean change: −0.20 ± 1.20 g/g) were observed the *L. acidophilus* KBL409 group (Table 2). There were no significant differences in the mean eGFR of both groups after 16 weeks of administration in the per protocol set. However, in the full analysis set, a significantly lower reduction of the mean eGFR was observed in the *L. acidophilus* KBL409 group than the placebo group (data not shown). The mean serum IS concentration in the *L. acidophilus* KBL409 group was significantly reduced after 16 weeks compared to the placebo group ($p = 0.0329$). However, *L. acidophilus* KBL409 administration had no effects on serum PCS and TMAO concentrations.

Effects of *L. acidophilus* KBL409 on the gut microbiota

To investigate the effects of *L. acidophilus* KBL409 on the gut microbiota, the fecal microbiome of participants was analyzed (Fig. 2). Alpha diversities did not significantly differ between the *L. acidophilus* KBL409 group and the placebo group (Fig. 2A). Beta diversities of the fecal microbiome between the baseline (0 week) and after 16 weeks of administration were clustered similarly in both the *L. acidophilus* KBL409 group and the placebo group (Fig. 2B). After 16 weeks, the abundance of the genus *Lactobacillus* ($p < 0.01$) was significantly increased, whereas the abundances of the genera *Blautia* ($p < 0.01$), *Megamonas* ($p < 0.05$), *Butyricicoccus* ($p < 0.01$), and *Lachnospiraceae* UCG-004 ($p < 0.05$) were significantly decreased in the *L. acidophilus* KBL409 group (Fig. 2C). Moreover, after 16 weeks, the significant increase in the abundance of the genus *Lactobacillus* ($p < 0.05$) and significant decrease in the genera *Blautia* ($p < 0.01$) and *Lachnospiraceae* UCG-004 ($p < 0.01$) were discovered in the *L. acidophilus* KBL409 group compared to the placebo group. Additionally, in a subgroup of participants in the *L. acidophilus* KBL409 group with a mean eGFR <60 mL/min/1.73 m^2, significant decreases were also observed in the abundances of the genera *Butyricicoccus* ($p < 0.01$) and *Lachnospiraceae* UCG-004 ($p < 0.01$) in the *L. acidophilus* KBL409 group compared to the placebo group (Fig. 2D).

Table 2 | Changes in kidney function indicators and uremic toxin concentrations during the intervention period

Data[a]	*L. acidophilus* KBL409 group ($n = 34$)				Placebo group ($n = 30$)				*P*-value
	Baseline	16 weeks	Change	*P*-value[b]	Baseline	16 weeks	Change	*P*-value[b]	
eGFR (mL/min/1.73 m^2)	62.03 ± 24.04	61.47 ± 25.73	−0.56 ± 7.47	0.6655	63.55 ± 24.19	60.08 ± 24.25	−3.46 ± 6.92	0.0104	0.1134[c]
Urine protein (mg/dL)	224.85 ± 299.97	196.43 ± 275.60	−28.42 ± 170.81	0.3390	205.12 ± 151.92	197.28 ± 152.15	−7.84 ± 154.38	0.7828	0.8665[d]
Urine creatinine (mg/dL)	113.12 ± 43.18	108.92 ± 74.62	−4.19 ± 82.55	0.7689	120.31 ± 65.42	133.97 ± 76.55	13.66 ± 76.50	0.3361	0.3429[d]
UPCR (g/g)	2.02 ± 2.22	1.82 ± 1.81	−0.20 ± 1.20	0.3305	1.91 ± 1.48	1.78 ± 1.58	−0.14 ± 0.81	0.3655	0.8296[d]
Serum PCS (mg/L)	4.92 ± 4.22	5.13 ± 5.50	0.21 ± 3.56	0.7324	5.98 ± 5.37	5.90 ± 5.01	−0.29 ± 3.61	0.6766	0.9605[d]
Serum IS (mg/L)	0.27 ± 0.18	0.23 ± 0.17	−0.03 ± 0.08	0.0226	0.22 ± 0.16	0.22 ± 0.17	0.00 ± 0.12	0.9880	0.0329[d]
Serum TMAO (μmol/L)	0.91 ± 0.91	0.91 ± 0.75	0.00 ± 0.99	1.0000	1.36 ± 1.53	1.22 ± 1.54	−0.14 ± 1.54	0.6301	0.9517[d]

[a]Data are presented as means ± SD.
[b]Intra-group comparisons were performed using a paired t-test.
[c]Inter-group comparisons in changes from baseline to 16 weeks were performed using a two-sample t-test.
[d]Inter-group comparisons in changes from baseline to 16 weeks were performed using the Wilcoxon rank-sum test.

Effects of *L. acidophilus* KBL409 on profiles of functional gene alterations related to the gut microbiota

In the *L. acidophilus* KBL409 group, significant reductions were observed in the activities of predicted metabolic pathways for phenylalanine, shikimate, tryptophan, and tyrosine biosynthesis after 16 weeks of administration (Fig. 3). A significant decrease in the expression of *tyr*A2, which encodes prephenate dehydrogenase, also identified, indicating alterations in tryptophan metabolism pathways associated with gut microbial compositions.

Correlations between specific gut bacteria and predicted genes in the *L. acidophilus* KBL409 group

Table 3 presents the correlations between specific gut bacteria and predicted genes related to uremic toxin synthesis pathways in the *L. acidophilus* KBL409 group. The abundances of the genera *Blautia*, *Butyricicoccus*, *Lachnospiraceae* UCG-004, and *Megamonas*, which significantly decreased after 16 weeks of *L. acidophilus* KBL409 administration, were positively correlated with various predicted genes involved in the biosynthesis and degradation of aromatic amino acids. These findings suggest that *L. acidophilus* KBL409 downregulated uremic toxin-producing pathways by reducing the abundances of specific gut bacteria.

Safety

During the entire intervention period, a total of 11 adverse events were reported in the *L. acidophilus* KBL409 group, including 10 mild cases and 1 moderate case (cholecystitis acute) (Table S2). Similarly, 11 mild adverse events were reported in the placebo group. Only two gastrointestinal disorders, including constipation and gastrointestinal disorders, were identified as the adverse events probably or possibly related to *L. acidophilus* KBL409 administration, respectively. All adverse events were resolved during the intervention period. Blood and urine samples were collected from participants during Visit 1 and Visit 5 for safety assessments. All safety parameters did not show a significant difference between the *L. acidophilus* KBL409 group and the placebo group.

Discussion

The metabolism-dependent pathway of the gut-kidney axis is critical for the uremic toxin production[31,32]. Initially, dietary patterns high in proteins and animal fats and low in resistant starches and fibers induce dysbiosis of gut microbiota, resulting in excessive production and accumulation of uremic toxins, including IS and PCS. These uremic toxins disrupt intestinal barriers and promote kidney inflammations with endotoxins[33]. Our previous studies showed that *L. acidophilus* KBL409 significantly upregulated the expression of gut tight-junction markers, such as zonula occludens (ZO)-1 and claudin-1, increased the diversity of cecum microbiota, and reduced NLR family pyrin domain containing 3 inflammasomes and kidney fibrosis markers such as alpha-1 type I collagen and fibronectin in an adenine-induced CKD mouse model[28–30]. In the present study, *L. acidophilus* KBL409 administration effectively reduced serum IS concentrations, as well as other kidney function indicators, including urine protein and urine creatinine (Table 2). These findings suggest that *L. acidophilus* KBL409 can serve as a probiotic microorganism for improving of kidney health.

Notably, there were no significant changes in eGFR in the *L. acidophilus* KBL409 group, indicating that *L. acidophilus* KBL409 administration can reduce serum IS concentrations without altering kidney functions (Table 2). However, significant reductions of other uremic toxins, such as PCS and TMAO, were not observed during the intervention period. Previous studies have reported that different probiotic strains exhibit varying capabilities to reduce uremic toxin levels[27]. However, our results only evaluated the effects of *L. acidophilus* KBL409 over a 16-week period. Further longitudinal studies in humans, incorporating various doses are necessary to assess the potential renoprotective effects of *L. acidophilus* KBL409.

Figure 2C suggests that *L. acidophilus* KBL409 can affect the abundances of specific gut bacteria, including the genera *Blautia*, *Butyricicoccus*, *Lachnospiraceae* UCG-004, and *Megamonas*.

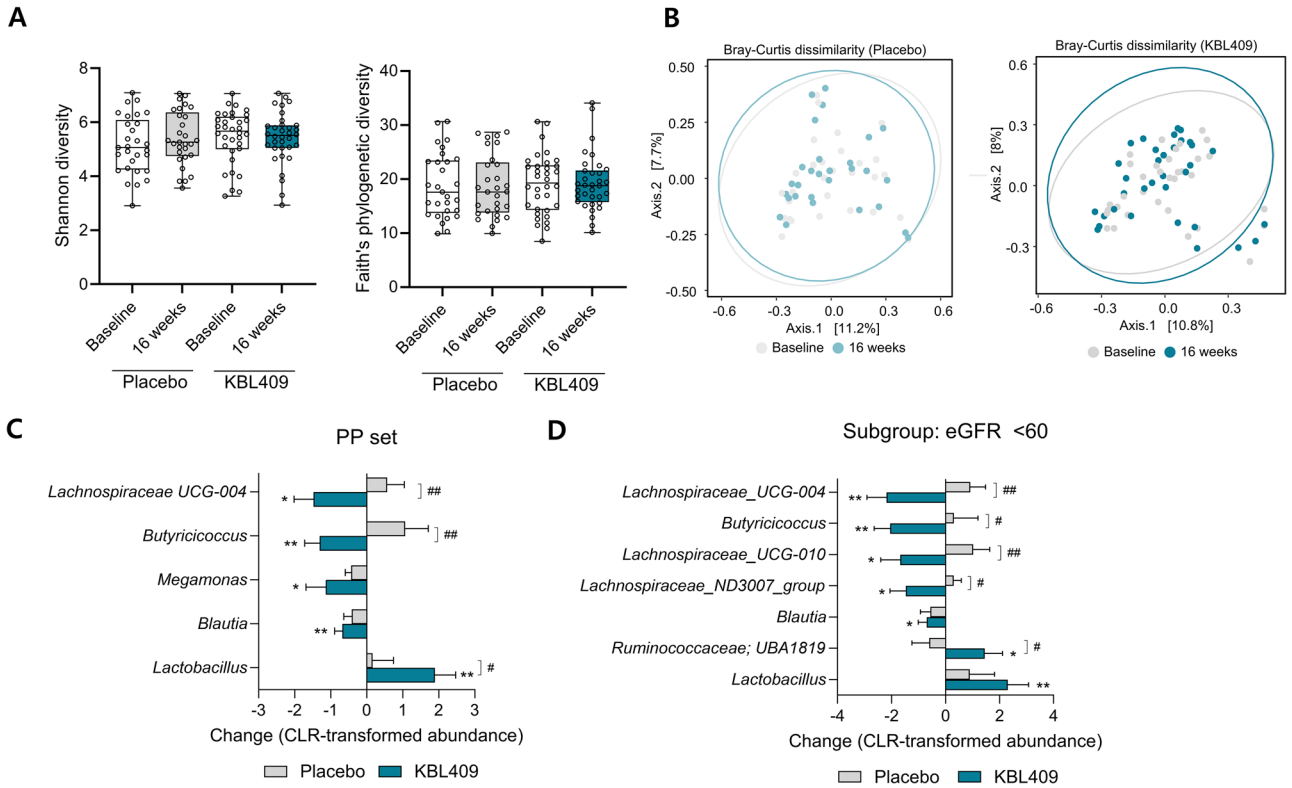

Fig. 2 | Effects of *L. acidophilus* KBL409 on the gut microbiota of participants in the per protocol set. A Alpha diversity indices. **B** Principal coordinates analysis plots based on the Bray–Curtis dissimilarity distances. **C** Relative abundances of specific gut bacteria in participants after 16 weeks of *L. acidophilus* KBL409 administration. **D** Relative abundances of specific gut bacteria in subgroup participants (the mean eGFR <60 mL/min/1.73 m^2) after 16 weeks of *L. acidophilus* KBL409 administration. Asterisks indicate statistical significance in intra-group comparisons ($^*p < 0.05$; $^{**}p < 0.01$; a paired t-test). Hashtags indicate statistical significance in inter-group comparisons in changes from baseline to 16 weeks ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$; a two-sample t-test).

Interestingly, participants with a mean eGFR <60 mL/min/1.73 m^2, indicating moderate impairment of CKD[34], also showed the decreases in the abundances of genera *Blautia*, *Butyricicoccus*, and *Lachnospiraceae* UCG-004 (Fig. 2D). These bacteria showed strong positive correlations with various predicted genes related to the biosynthesis and degradation of aromatic amino acids, including phenylalanine, tryptophan, and tyrosine (Table 3). Consequently, significant decreases in predicted metabolic pathways of aromatic amino acids were observed in the *L. acidophilus* KBL409 group after 16 weeks of administration (Fig. 3). Aromatic amino acids are key precursors for uremic toxins[35]. Changes in gut microbial composition due to *L. acidophilus* KBL409 administration could play crucial roles in reducing serum IS concentrations. However, to establish a clear causal relationship between *L. acidophilus* KBL409-mediated modulation of gut microbiota and the reduction of uremic toxins, comprehensive profiling of uremic toxin precursors in fecal, blood, and urine samples is necessary. Further studies with precise tracking of gut microbiota and metabolites related to uremic toxin production should be planned to fully elucidate the mechanisms of *L. acidophilus* KBL409 in preventing CKD via the gut-kidney axis.

Our findings are consistent with previous research emphasizing the important role of gut microbiota in the accumulation of uremic toxins[8]. However, the relatively small sample size of this study limited the statistical power to identify the effects of *L. acidophilus* KBL409 on major kidney function indicators, such as eGFR and UPCR. Additionally, the effects of *L. acidophilus* KBL409 on uremic toxins may have been underestimated because, in this study, only participants who have lower serum concentrations of uremic toxins were enrolled. Furthermore, the results of the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)2 analyses provided only functional predictions based on changes in gut microbiota. Further studies are necessary to evaluate the preventive or therapeutic potential and elucidate the specific mechanisms of *L. acidophilus* KBL409.

In conclusion, *L. acidophilus* KBL409 significantly reduced serum IS concentrations in study participants and altered the gut microbiota by decreasing the abundances of the genera *Blautia*, *Butyricicoccus, Lachnospiraceae* UCG-004, and *Megamonas*. These bacteria showed positive correlations with the activities of predicted functional genes related to uremic toxin synthesis pathways, indicating that *L. acidophilus* KBL409 effectively reduced serum IS within the gut-kidney axis. Especially, the effects of gut microbial alterations due to *L. acidophilus* KBL409 on serum IS concentrations were notable because the *L. acidophilus* KBL409 group showed the clear high protein-dietary pattern during this study. Our findings suggest that *L. acidophilus* KBL409 could be a safe and promising probiotic for improving kidney health. Further human studies involving patients with severe CKD, utilizing different dose- and time-dependent approaches, will be necessary to expand the applications of *L. acidophilus* KBL409 in the treatment of CKD.

Methods

Subjects

This study was conducted in accordance with Korean Good Clinical Practice (KGCP) Guidelines and the Declaration of Helsinki. All participants provided informed consent, and the study protocol was approved by the institutional review boards of Severance Hospital, Yonsei University Health System (No. 4-2019-0763), SMG-SNU Boramae Medical Center (No. 20-2019-59), and National Health Insurance Service Ilsan Hospital (No. NHIMC 2019-08-002-020).

A total of 92 individuals visited the study sites during the study period (November 2019 to November 2020) and 82 participants met the inclusion criteria (Fig. 1). The inclusion criteria for participants were: (1) aged 19 or

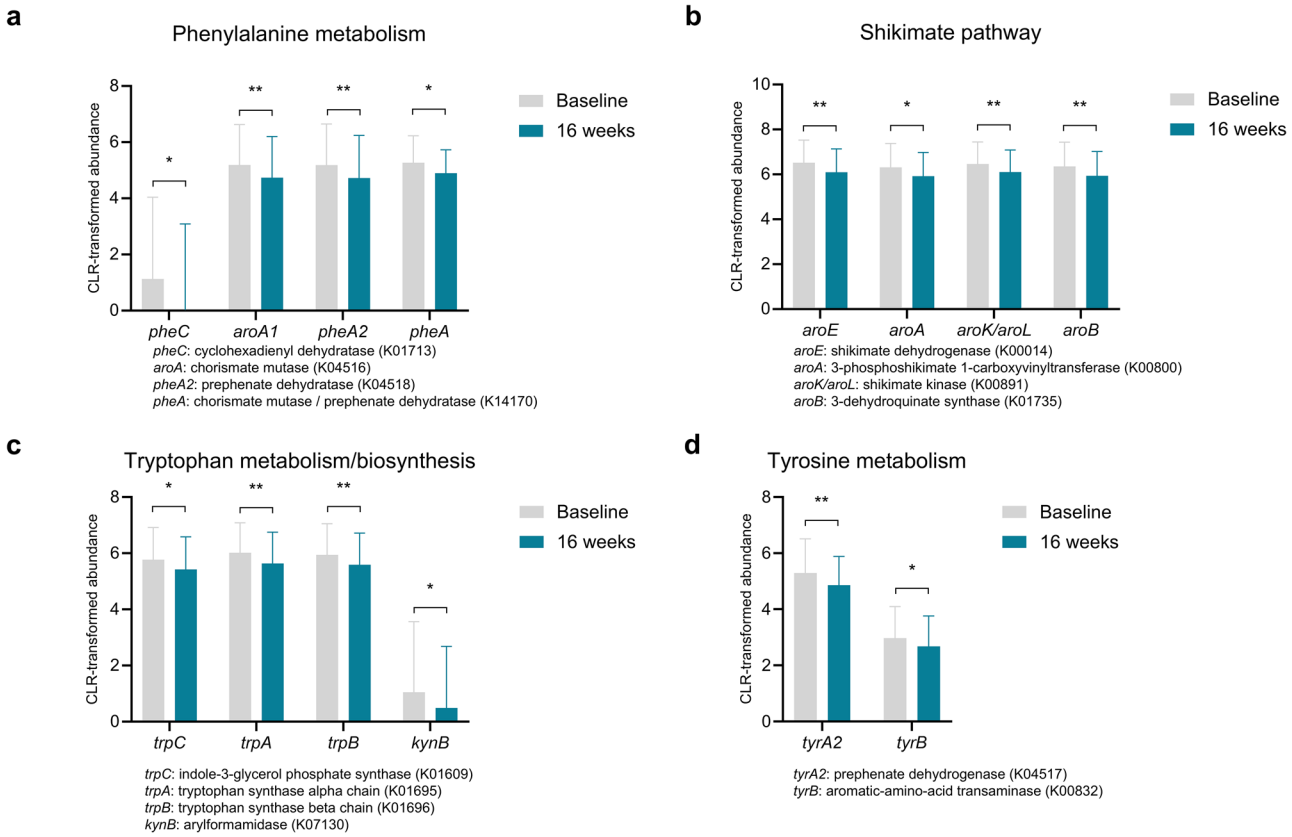

Fig. 3 | Profiles of functional gene alterations related to the gut microbiota of participants with *L. acidophilus* KBL409 administration in the per protocol set. **a** Phenylalanine metabolism. **b** Shikimate pathway. **c** Tryptophan metabolism/biosynthesis. **d** Tyrosine metabolism. Asterisks indicate statistical significance (*$p < 0.05$; **$p < 0.01$; a paired t-test).

Table 3 | Spearman's correlation coefficients among specific gut bacteria and predicted genes related to uremic toxin synthesis pathways in the *L. acidophilus* KBL409 group

Genus	Gene (encoded protein)	Coefficient	*P*-value
Blautia	*aroF*, *aroG*, *aroH* (3-deoxy-7-phosphoheptulonate synthase)	0.2587	0.0331
	tyrB (aromatic amino acid transaminase)	0.3151	0.0089
	aroD (3-dehydroquinate dehydratase I)	0.4186	0.0004
Butyricicoccus	*pheA2* (prephenate dehydratase)	0.2395	0.0492
	aroA1, *aroA* (chorismate mutase)	0.2457	0.0434
	aroD (3-dehydroquinate dehydratase I)	0.2564	0.0348
	pheC (cyclohexadienyl dehydratase)	0.2743	0.0236
	tyrB (aromatic amino acid transaminase)	0.3663	0.0021
Lachnospiraceae UCG-004	*aroD* (3-dehydroquinate dehydratase I)	0.3205	0.0077
	trpA (tryptophan synthase alpha chain)	0.3312	0.0058
	trpB (tryptophan synthase beta chain)	0.3349	0.0052
	kynB (arylformamidase)	0.3549	0.0030
	pheC (cyclohexadienyl dehydratase)	0.3804	0.0014
Megamonas	*trpB* (tryptophan synthase beta chain)	0.4119	0.0005
	trpG (anthranilate synthase component II)	0.4186	0.0004
	pheA2 (prephenate dehydratase)	0.4318	0.0002
	kynB (arylformamidase)	0.4383	0.0002
	tyrB (aromatic amino acid transaminase)	0.5455	<0.0001

older; (2) eGFR >30 mL/min/1.73 m^2; (3) urine protein to creatinine ratio (UPCR) > 0.5 g/g; (4) voluntarily agreed to participate in this study with written informed consent provided. The major exclusion criteria were: (1) history of renal dialysis, kidney transplantation, or glomerulonephritis; (2) use of renal medications including Kremezin®, Renamezin®, and tolvaptan within 4 weeks prior to Visit 1; (3) use of immunosuppressants; (4) use of systemic antibiotics or probiotics within two weeks prior to Visit 1; (5) regular consumption of fermented milk (more than four times per week);

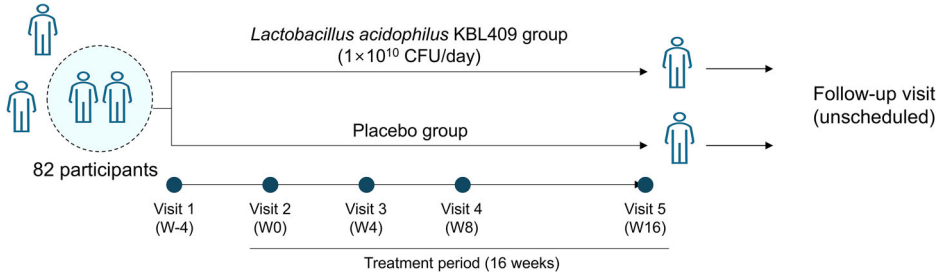

Fig. 4 | Study design.

(6) allergic reactions to functional food ingredients; (7) history of thyroid diseases; (8) levels of aspartate aminotransferase or alanine aminotransferase more than three times of the upper limit of normal (40 IU/L); (9) participation in another clinical study within one month or willingness to participation in another clinical study after this study; (10) pregnancy or breastfeeding; (11) any conditions deemed inappropriate by investigators.

Study design

This study is a multi-center, randomized, double-blind, placebo-controlled study to assess the efficacy of *L. acidophilus* KBL409 in reducing uremic toxin levels among study participants. A total of 82 participants were assigned randomly to either the *L. acidophilus* KBL409 or the placebo group at a 1:1 ratio using SAS v.9.4 (SAS Institute, Cary, NC, USA) (Fig. 4). All participants received either *L. acidophilus* KBL409 or placebo capsules, which were labeled according to the randomization table, at 4-week intervals. To maintain the integrity of the participant allocation process, both investigators and participants were blinded to the allocation codes until the study was completed.

Participants in the *L. acidophilus* KBL409 group received a capsule containing 1×10^{10} colony-forming units of *L. acidophilus* KBL409 daily for 30 min before a meal. Placebo capsules, mainly composed of dextrin, were prepared to match the appearance, flavor, and color of *L. acidophilus* KBL409 capsules. All capsules were stored at 2–8 °C until use.

The study consisted of a screening visit (Visit 1) and four additional visits (Visit 2–5) during the 16-week intervention period (Fig. 4). During Visit 3–5, a 7-day visit window was allowed for participants. Follow-up visits were performed as necessary. Participants were advised to maintain appropriate lifestyles, including a low-protein diet, moderate exercise, controlled alcohol consumption, weight management, and smoking cessation. Demographic characteristics, medical histories, clinical characteristics, and lifestyle details of participants were documented at Visit 1. Additionally, eGFR and UPCR measurements were conducted to determine the enrollment of study participants. During Visit 2–5, dietary surveys by 24-h recall method were completed for eligible participants.

Blood and urine samples were collected from participants for further analyses of biomarkers, including eGFR, UPCR, uremic toxins, and cytokines. Briefly, 17.0 mL of blood was collected from each participant using BD Vacutainer SST II Advance Tubes (BD Biosciences, San Jose, CA, USA). The collected blood samples were inverted several times, incubated at room temperature for 30 min, and centrifuged at 3000 rpm for 10 min. The supernatant, which is the separated serum, was collected into cryotubes and stored at −70 °C until use. Additionally, 1.0 mL of midstream urine was collected from each participant into cryotubes and stored at −70 °C until use.

Fecal samples were self-collected by participants using DNA/RNA Shield-Fecal Collection Tubes (Zymo Research, Irvine, CA, USA) and immediately stored at −20 °C. During Visit 2 and Visit 5, frozen fecal samples were gathered and stored at −70 °C until use.

Analysis of eGFR and UPCR

To assess eGFR, and UPCR, serum and urine samples were transferred to SCL Healthcare (Yongin-si, Gyeonggi-do, Republic of Korea), the central laboratory for this study, using a cold-chain transport route. Subsequently, these samples were analyzed in accordance with the central laboratory's standard procedures.

Analysis of uremic toxins

Standard stock solutions of IS (Sigma-Aldrich, St. Louis, MO, USA), PCS (MedChemExpress, Monmouth Junction, NJ, USA), and TMAO (Sigma-Aldrich) were prepared in 50% methanol (Sigma-Aldrich) and stored at −70 °C until use. All calibration standards and quality control samples were freshly prepared on the day of analysis.

To prepare samples for the analysis of uremic toxins, 30 µL of the internal standard solution, 300 µL of methanol, and 30 µL of serum were mixed and incubated at room temperature for 20 min. The mixture was centrifuged at 13,523 × *g* at 4 °C for 5 min, and 100 µL of the supernatant was collected in an autosampler vial. Subsequently, 5 µL of the supernatant were injected into the high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) system, which consisted of an Agilent 1200 Series Analytical LC System (Agilent Technologies, Waldbronn, Germany) and an API 5000 Triple Quadrupole Mass Spectrometer (AB SCIEX, Framingham, MA, USA). Separation was preformed using a reverse-phase Gemini C18 column (5 µm; 2.0 × 150 mm^2). HPLC-grade water containing 0.1% formic acid (Merck, Darmstadt, Germany) and liquid chromatography-grade acetonitrile (Sigma-Aldrich) were used as mobile phase A and mobile phase B, respectively. Isocratic conditions with 60% mobile phase B were applied. HPLC-MS/MS analyses for target uremic toxins were performed in multiple reaction monitoring mode under the following conditions: 0.2 mL/min flow rate, 15 min total running time, 40 °C column over temperature, and 8 °C autosampler temperature.

Analysis of gut microbiota

The gut microbiota of participants was analyzed using fecal samples, as previously described with some modifications[36]. Initially, total bacterial DNA was extracted using a FastDNA Stool Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The V3-V4 region of the 16S ribosomal RNA (16S rRNA)-coding gene was amplified using the following primers: 341 F (5′-CCTACGGGNGGCWGCAG-3′) and 805 R (5′- GACTACHVGGGTATCTAATCC-3′)[37]. An Illumina MiSeq platform (Illumina, San Diego, CA, USA) and a MiSeq Reagent Kit v. 3 (Illumina) were used for the sequencing process of barcoded amplicons. FastQC v. 0.11.9 (Bioinformatics Group of Babraham Institute; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Trimmomatic v. 0.38 (USEDEL lab; https://github.com/usadellab/Trimmomatic)[38] were applied for the manipulations of raw reads, including the removal of primer sequences and quality control. Reads below a Q33 quality score were excluded from analyses. Quantitative Insights into Microbial Ecology (QIIME) 2 v. 2024.5 (QIIME 2 Development Team; https://qiime2.org)[39], using the Divisive Amplicon Denoising Algorithm 2, was applied for demultiplexing, denoising, and chimera checking of reads. Subsequently, amplicon sequence variants (ASVs) were generated and classified using the SILVA v. 138.1 database (SILVA rRNA database project; https://www.arb-silva.de)[40]. ASVs with fewer than 500 reads or a minimum frequency <20% were excluded to reduce noise. Alpha diversity indices, including Faith's

phylogenetic diversity and Shannon diversity, were calculated using the q2-diversity function in QIIME 2 v. 2024.5. Principal coordinates analysis (PCoA) plots with the Bray–Curtis dissimilarity distances for beta diversities were also suggested using the phyloseq R package in Bioconductor v. 3.20 (The Bioconductor Project; https://www.bioconductor.org/packages/release/bioc/html/phyloseq.html)[41]. Profiles of functional gene alterations related to gut microbiota were analyzed using PICRUSt 2 v. 2.5.3 (The Huttenhower Lab; https://huttenhower.sph.harvard.edu/picrust/)[42] with the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologous gene family database (Kanehisa Laboratories, Kyoto University; https://www.genome.jp/kegg/)[43].

Participant size and statistical analysis

The appropriate participant size for was calculated based on the results of a previous study[44]. UPCR was used as the variable to calculate the number of participants. To achieve 80% power with an alpha error probability of 0.05 and a beta error probability of 0.2, a minimum of 32 participants were required in each group. The target enrollment was set at 40 participants per group to adjust for a potential 20% drop-out rate.

All statistical analyses for the per protocol set were performed using SAS v. 9.4 (SAS Institute). Categorical and continuous variables are presented as frequencies with proportions or as means ± standard deviations (SDs), respectively. The chi-square test or Fisher's exact test were used for inter-group comparisons of categorical variables. For continuous variables, a paired t-test for intra-group comparisons or a two-sample t-test or the Wilcoxon rank-sum test for inter-group comparisons were performed, as appropriate. A P-value (p) <0.05 was considered statistically significant. The centered log-ratio transformation was applied to normalize gut microbiota abundances. Spearman correlation coefficients among specific gut bacteria and predicted genes related to uremic toxin synthesis pathways were calculated using the rcorr function in R v. 4.1.2 (R Core Team; https://www.r-project.org).

Data availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Krukowski, H. et al. Gut microbiome studies in CKD: opportunities, pitfalls and therapeutic potential. *Nat. Rev. Nephrol.* **19**, 87–101 (2023).
2. Chesnaye, N. C., Ortiz, A., Zoccali, C., Stel, V. S. & Jager, K. J. The impact of population ageing on the burden of chronic kidney disease. *Nat. Rev. Nephrol.* **2**, 569–585 (2024).
3. Francis, A. et al. Chronic kidney disease and the global public health agenda: an international consensus. *Nat. Rev. Nephrol.* **20**, 473–485 (2024).
4. Zoccali, C. et al. The systemic nature of CKD. *Nat. Rev. Nephrol.* **13**, 344–358 (2017).
5. Hung, S. C., Kuo, K. L., Wu, C. C. & Tarng, D. C. Indoxyl sulfate: a novel cardiovascular risk factor in chronic kidney disease. *J. Am. Heart Assoc.* **6**, e005022 (2017).
6. Wang, M. et al. The gut microbial metabolite trimethylamine N-oxide, incident CKD, and kidney function decline. *J. Am. Soc. Nephrol.* **35**, 749–760 (2024).
7. Rosner, M. H. et al. Classification of uremic toxins and their role in kidney failure. *Clin. J. Am. Soc. Nephrol.* **16**, 1918–1928 (2021).
8. Gryp, T. et al. Gut microbiota generation of protein-bound uremic toxins and related metabolites is not altered at different stages of chronic kidney disease. *Kidney Int.* **97**, 1230–1242 (2020).
9. Vanholder, R. et al. Metabolomics to identify unclassified uremic toxins: a comprehensive literature review. *Kidney Med.* **7**, 100955 (2024).
10. Chakaroun, R. M., Olsson, L. M. & Bäckhed, F. The potential of tailoring the gut microbiome to prevent and treat cardiometabolic disease. *Nat. Rev. Cardiol.* **20**, 217–235 (2023).
11. Koh, A. & Bäckhed, F. From association to causality: the role of the gut microbiota and its functional products on host metabolism. *Mol. Cell* **78**, 584–596 (2020).
12. Fan, Y. & Pedersen, O. Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* **19**, 55–71 (2021).
13. Mirmiran, P., Yuzbashian, E., Asghari, G., Sarverzadeh, S. & Azizi, F. Dietary fibre intake in relation to the risk of incident chronic kidney disease. *Br. J. Nutr.* **119**, 479–485 (2018).
14. Stavropoulou, E. et al. Focus on the gut-kidney axis in health and disease. *Front. Med.* **7**, 620102 (2021).
15. Yang, T., Richards, E. M., Pepine, C. J. & Raizada, M. K. The gut microbiota and the brain-gut-kidney axis in hypertension and chronic kidney disease. *Nat. Rev. Nephrol.* **14**, 442–456 (2018).
16. Roager, H. M. & Licht, T. R. Microbial tryptophan catabolites in health and disease. *Nat. Commun.* **9**, 3294 (2018).
17. Graboski, A. L. et al. Mechanism-based inhibition of gut microbial tryptophanases reduces serum indoxyl sulfate. *Cell. Chem. Biol.* **30**, 1402–1413 (2023).
18. Wang, X. et al. Aberrant gut microbiota alters host metabolome and impacts renal failure in humans and rodents. *Gut* **69**, 2131–2142 (2020).
19. Devlin, A. S. et al. Modulation of a circulating uremic solute via rational genetic manipulation of the gut microbiota. *Cell Host Microbe* **20**, 709–715 (2016).
20. Kim, H. Y. et al. Uremic toxin indoxyl sulfate induces trained immunity via the AhR-dependent arachidonic acid pathway in end-stage renal disease (ESRD). *Elife* **12**, RP87316 (2024).
21. Barisione, C. et al. Indoxyl sulphate-initiated activation of cardiac fibroblasts is modulated by aryl hydrocarbon receptor and nuclear factor-erythroid-2-related factor 2. *J. Cell. Mol. Med.* **28**, e18192 (2024).
22. Lekawanvijit, S. et al. Does indoxyl sulfate, a uraemic toxin, have direct effects on cardiac fibroblasts and myocytes? *Eur. Heart. J.* **31**, 1771–1779 (2010).
23. Rossi, M. et al. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): a randomized trial. *Clin. J. Am. Soc. Nephrol.* **11**, 223–231 (2016).
24. Ranganathan, N. et al. Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada. *Curr. Med. Res. Opin.* **25**, 1919–1930 (2009).
25. Sohn, M. B. et al. Pilot studies in CKD consortium. targeting gut microbiome with prebiotic in patients with CKD: The TarGut-CKD study. *Kidney Int. Rep.* **9**, 671–685 (2023).
26. Fang, C. Y. et al. Selection of uremic toxin-reducing probiotics in vitro and in vivo. *J. Funct. Foods* **7**, 407–415 (2014).
27. Vacca, M. et al. In vitro selection of probiotics, prebiotics, and antioxidants to develop an innovative synbiotic (NatuREN G) and resting its effect in reducing uremic toxins in fecal batches from CKD patients. *Microorganisms* **9**, 1316 (2021).
28. Kim, H. et al. *Lactobacillus acidophilus* KBL409 reduces kidney fibrosis via immune modulatory effects in mice with chronic kidney disease. *Mol. Nutr. Food Res.* **66**, e2101105 (2021).
29. Kim, M. J. et al. Effects of *Lactobacillus acidophilus* KBL409 administration on uremic toxins, *Korean*. *J. Microbiol.* **60**, 161–168 (2024).
30. Park, J. et al. *Lactobacillus acidophilus* KBL409 protects against kidney injury via improving mitochondrial function with chronic kidney disease. *Eur. J. Nutr.* **63**, 2121–2135 (2024).
31. Glorieux, G., Nigam, S. K., Vanholder, R. & Verbeke, F. Role of the microbiome in gut-heart-kidney cross talk. *Circul. Res.* **132**, 1064–1083 (2023).

32. Evenepoel, P., Meijers, B. K., Bammens, B. R. & Verbeke, K. Uremic toxins originating from colonic microbial metabolism. *Kidney Int. Suppl.* **114**, S12–S19 (2009).
33. Lau, W. L., Kalantar-Zadeh, K. & Vaziri, N. D. The gut as a source of inflammation in chronic kidney disease. *Nephron* **130**, 92–98 (2015).
34. Levin, A. & Stevens, P. E. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int.* **85**, 49–61 (2014).
35. Barba, C. et al. A low aromatic amino-acid diet improves renal function and prevent kidney fibrosis in mice with chronic kidney disease. *Sci. Rep.* **11**, 19184 (2021).
36. Yoon, H. S. et al. *Akkermansia muciniphila* secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. *Nat. Microbiol.* **6**, 563–573 (2021).
37. Singer, E. et al. High-resolution phylogenetic microbial community profiling. *ISME J.* **10**, 2020–2032 (2016).
38. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
39. Bolyen, E. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
40. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013).
41. McMurdie, P. J. & Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS ONE* **8**, e61217 (2013).
42. Douglas, G. M. et al. PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* **38**, 685–688 (2020).
43. Ogata, H. et al. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **27**, 29–34 (1999).
44. Oblak, M., Mlinšek, G., Kandus, A., Buturović-Ponikvar, J. & Arnol, M. Paricalcitol versus placebo for reduction of proteinuria in kidney transplant recipients: a double-blind, randomized controlled trial. *Transpl. Int.* **31**, 1391–1404 (2018).

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Author contributions

S.J.J.: Conceptualization, formal analysis, investigation, methodology, visualization, writing—original draft. S.P.: Conceptualization, investigation, methodology, supervision, writing—original draft, writing—review and editing. K.L.: Formal analysis, investigation, methodology. W.-K.K.: Investigation, methodology. S.H.M.: Investigation, methodology. B.-R.C.: Investigation, methodology. C.L.: Methodology, Writing—original draft, writing – review & editing. H.L.: Conceptualization, funding acquisition. T.-W.N.: Conceptualization, investigation, funding acquisition, project administration. G.K.: Conceptualization, funding acquisition, resources, supervision.

Competing interests

G.K. is the chief executive officer of KoBioLabs, Inc. K.L., S.H.M., and B-R.C. are employees of KoBioLabs, Inc. H.L. is the chief executive officer of weBiom Inc. S.J.J. and S.P. are employees of weBiom Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Additional information

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Correspondence and requests for materials should be addressed to SungJun Park or GwangPyo Ko.