Gut microbiota dynamics and uraemic toxins: one size does not fit all

In the recent paper by Chu and colleagues,[1] the potential role of microbiota-related metabolites in the progression of non-alcoholic fatty liver disease is discussed. This topic has been studied in the context of chronic kidney disease (CKD), characterised by changes in gut microbiota composition,[2] accumulation of microbiota-derived metabolites,[3] interruption of intestinal barrier function and chronic inflammation.[4] In line with this, we focused, in a cohort of 17 patients with end-stage kidney disease (ESKD), on the role of gut microbiota in the generation of precursors of specific uraemic toxins which are associated with negative

outcomes in these patients.[5] By collecting multiple samples over time, assessment of variability within and between patients in relation to disease progress and clinical variables was possible. Faecal and serum samples were collected at eight time-points over a 4-month period (online supplementary table 1). Uraemic metabolites and microbial profiling were determined by HPLC and 16S rRNA amplicon sequencing, respectively (see Supplementary data). Variation in microbial profiles of patients with ESKD was compared with that of 1106 subjects from a population-based cohort, the Flemish Gut Flora Project (FGFP),[6] which have a similar genetic and environmental background as well as to a subset of age-matched controls of comparable health status (n=32).

In this longitudinal study, within-patient analyses showed that variations in peripheral levels of *p*-cresyl conjugates (the composite of *p*-cresyl sulfate (pCS)/

glucuronide (pCG); *p*C), indoxyl sulfate (IxS), indole acetic acid and creatinine[7] significantly correlated with faecal microbial community dissimilarity (at 0.05 level after Benjamini-Hochberg correction). Moreover, the composition of the gut microbiota was found to be diverse among patients with ESKD without a common microbial signature. A significantly higher variability of the patients' microbiome was observed in comparison to average subject-to-subject differences, even when matching for age and health status (both p<0.0001) (online supplementary figure S1). Projecting the patients' samples on the PCoA plot of the FGFP confirmed that these patients do not cluster in a specific area but rather are dispersed over the entire space of the control population (online supplementary figure S2).

Covariate analyses of the intestinal microbiota composition in ESKD resulted in non-redundant parameters

that significantly correlated with the overall composition, with length of scaled arrows reflecting correlation as depicted in figure 1 (list of covariates in online supplementary table 2 and figure S3). When focusing on the relationships between uraemic retention molecules and gut microbiota, significant correlations of two main uraemic toxins, IxS and *p*C, to the overall bacterial community composition (p<0.05 after multiple testing correction) stand out. Specifically, since they are associated with contrasting types of gut microbiota, as the arrows for IxS and *p*C pointed into an opposite direction. This, for the first time, provides a possible working hypothesis on the reported discordant effects of prebiotics, probiotics and synbiotics on circulating levels of these two toxins.[8–10]

When further comparing samples of patients with highest *p*C and lowest IxS to samples with lowest *p*C and highest
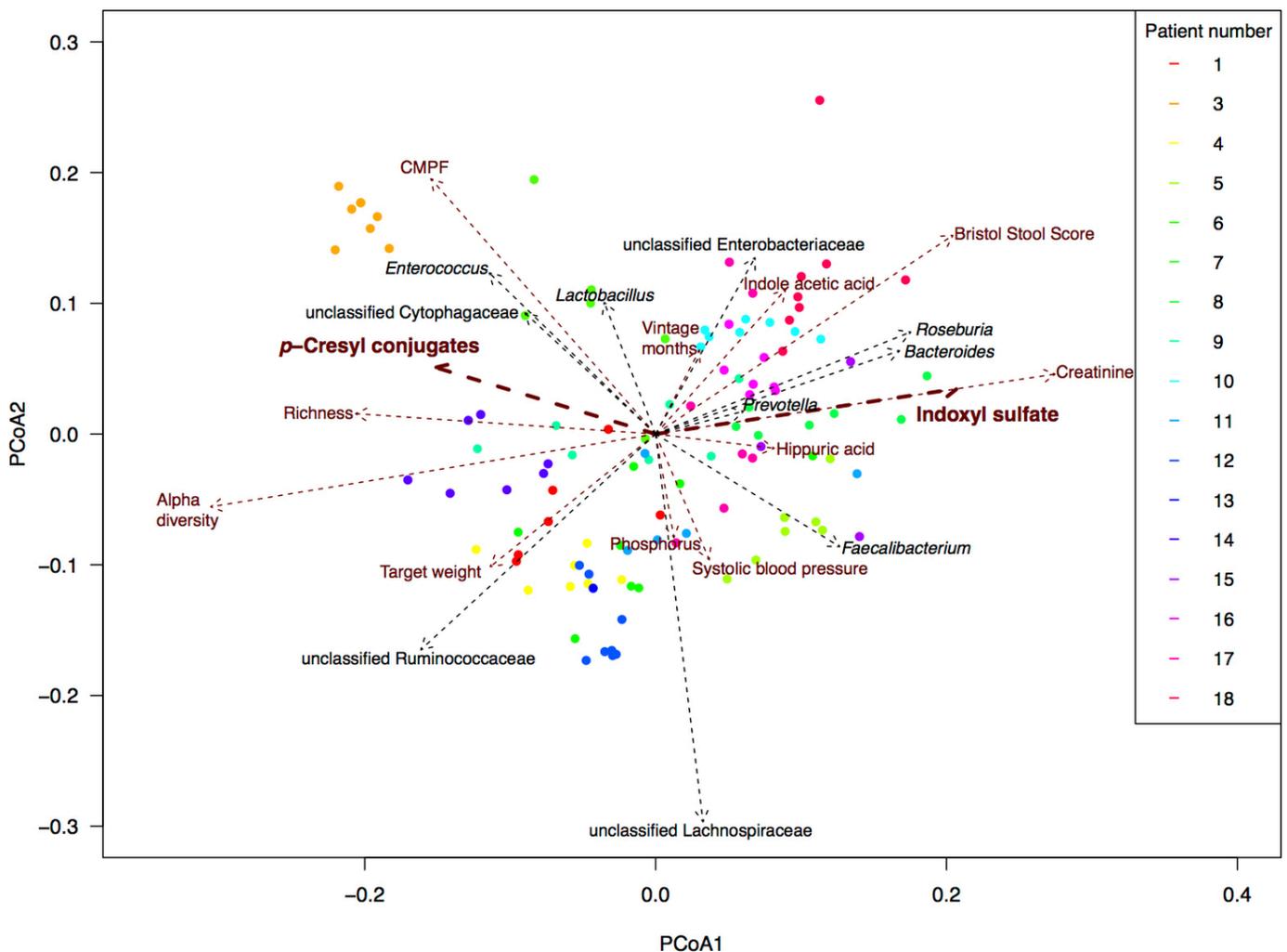
Figure 1 Main covariates of the faecal microbiota composition of patients with ESKD. Final selected numeric metadata in addition to top 10 taxa correlating with PCoA eigenvectors (ie, with overall community composition). Biplot computed with Bray Curtis dissimilarity on rarefied read counts. Length of arrows reflects correlation with overall community composition. Per patient, a different colour is used. CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; ESKD, end-stage kidney disease.
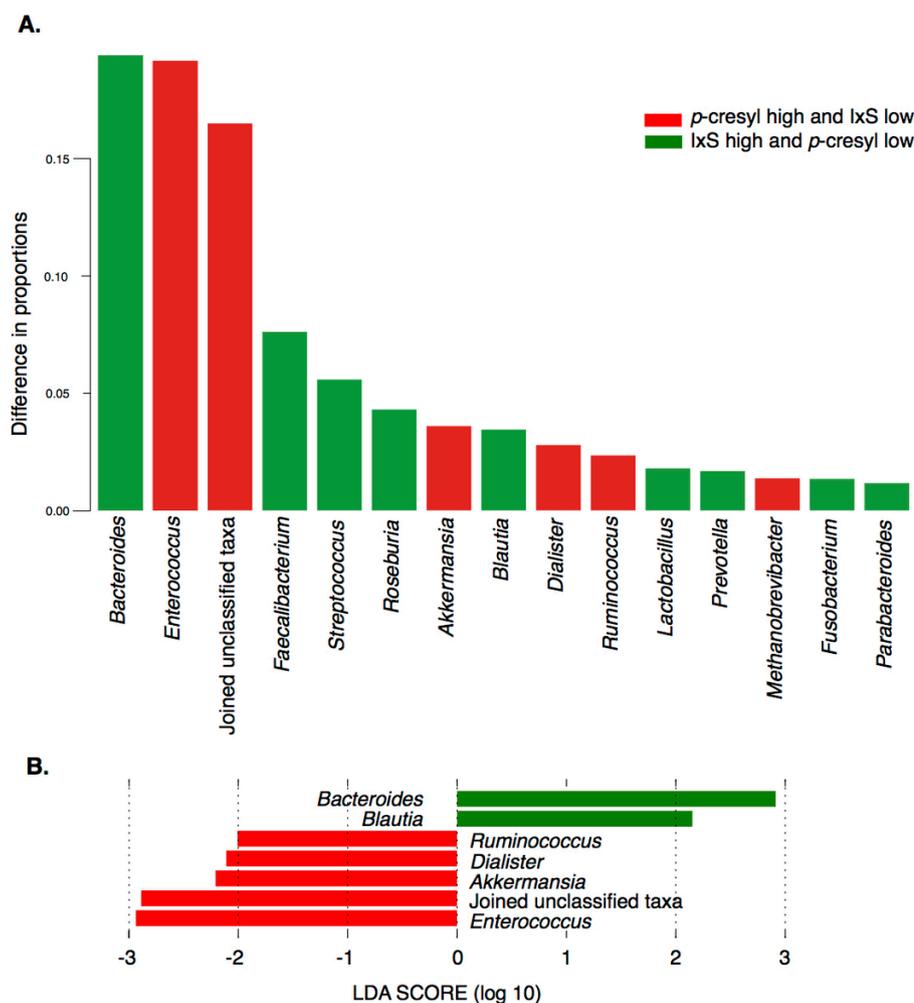
Figure 2 Associations between *p*-cresyl conjugates and indoxyl sulfate and intestinal microbiota, subcohort analysis. Faecal microbiota composition of samples with highest *p*-cresyl (*p*CS+*p*CG) and lowest IxS concentrations (group 1 in red) was compared with that of samples with highest IxS and lowest *p*C concentrations (group 2 in green). (A): The top 15 taxa with the largest difference between the two groups. Group-specific taxon proportion vectors were obtained by fitting the Dirichlet Multinomial distribution to each sample set (using R package HMP, function DM.MoM). The HMP package function Xmcupo.sevsample (Generalised Wald-type statistics) was used to compute whether the difference between the two taxon proportion vectors (highest *p*C/lowest IxS vs lowest *p*C/highest IxS) was significant (p<0.0001). (B) Effect sizes of genera that differed significantly between the datasets using LDA effect size (LEfSe). The length of the bar represents a log10 transformed LDA score. The colours represent in which group those taxa were found to be more abundant compared with the other group. Absolute values of the effect sizes should be used to interpret the scale of the difference between both groups. LDA, linear discriminant analysis.

IxS serum concentrations in this cohort, the microbial composition of their faecal samples differed significantly. Taxon proportions that differed between both groups are visualised in figure 2A. The LEfSe method confirmed that both datasets were different and identified in total six significantly different taxa together with their effect sizes (figure 2B), all six overlapping with the top 10 taxa that we identified earlier.

Our results illustrate the implications of gut microbiota dynamics on chronic disease and underscore the potential difficulties with attempts to alter circulating levels of intestinally generated uraemic toxins and their corresponding toxicity through specific microbiota modulation. Nevertheless, six taxa are identified and can now be explored as microbial targets to lower uraemic toxin concentrations and to improve outcome of patients with CKD.

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REFERENCES

1 Chu H, Duan Y, Yang L, *et al*. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut* 2019;68:359–70.
2 Vaziri ND, Wong J, Pahl M, *et al*. Chronic kidney disease alters intestinal microbial flora. *Kidney Int* 2013;83:308–15.
3 Vanholder R, Glorieux G. Gut-derived metabolites and chronic kidney disease: the Forest (F)or the trees? *Clin J Am Soc Nephrol* 2018;13:1311–3.
4 Anders HJ, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int* 2013;83:1010–6.
5 Barreto FC, Barreto DV, Liabeuf S, *et al*. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clin J Am Soc Nephrol* 2009;4:1551–8.
6 Falony G, Joossens M, Vieira-Silva S, *et al*. Population-level analysis of gut microbiome variation. *Science* 2016;352:560–4.
7 Eloot S, Van Biesen W, Roels S, *et al*. Spontaneous variability of pre-dialysis concentrations of uremic toxins over time in stable hemodialysis patients. *PLoS One* 2017;12:e0186010.
8 Meijers BK, De Preter V, Verbeke K, *et al*. p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin. *Nephrol Dial Transplant* 2010;25:219–24.
9 Hida M, Aiba Y, Sawamura S, *et al*. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* 1996;74:349–55.
10 Rossi M, Johnson DW, Morrison M, *et al*. Synbiotics Easing Renal Failure by Improving Gut Microbiology (SYNERGY): a randomized trial. *Clin J Am Soc Nephrol* 2016;11:223–31.