Should we integrate the gut microbiota composition to manage idiopathic nephrotic syndrome?

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Idiopathic nephrotic syndrome (INS) is the most frequent glomerular disease in childhood. There is overwhelming evidence that INS is immune mediated. INS is mainly attributed to the impaired function of T cells resulting in increased plasma lymphocyte-derived permeability factors even if the precise mechanisms remain unknown. Unfortunately, none of the available drugs (corticosteroids, calcineurin inhibitors, mycophenolate mofetil, levamisole and B cell–targeted drugs) proposed by the latest clinical practice recommendations lead to persistent remission in every patient [1, 2]. Today, INS in children is classified based on response to corticosteroid treatment. Although 90% of children exhibit a favorable response to steroids, only 25% of these children will effectively be cured and will not undergo further relapses after an initial course of steroids. Therefore, one of the major current challenges in INS is to identify which patients will have a persistent response to therapy, and which patients will relapse [3]. Indeed, using classical indicators such as clinical parameters or renal biopsies does not help in the prediction of the individual response to treatment. Our limited knowledge of the pathophysiological mechanisms underlying this glomerular disease is a major barrier to developing a successful therapeutic intervention.

Hence, there is an urgent need for fundamental and translational studies to understand underlying disease mechanisms, and identify specific biomarkers and therapeutic targets for further testing in clinical trials. The ultimate goal will be to propose a precision medicine to individualize diagnosis and treatment of INS. The identification of this precision medicine relies on data provided by several multi-omics fields that have sprung in the last years. Genomics (which studies the sequence of the whole genome), transcriptomics (which deals with the full set of transcripts), metabolomics (which analyses all low molecular weight compounds) and proteomics (which studies the entire proteome of a cell, tissue or organism) in combination with machine learning tools show promise in defining homogeneous subgroups within glomerular diseases. Using such an approach, Hodgkin et al. were able to sort adults and children with INS of the focal segmental glomerulosclerosis or minimal change disease into three clusters associated with clinical evolution (proteinuria remission, disease progression and renal function decline) [4]. Many research studies are currently ongoing on this topic, but until now very few studies have focused on the role of the gut microbiota in this disease. As a consequence, no feces collections were planned in

the initial design of the NEPTUNE cohort (Nephrotic Syndrome Study Network; a North American multicenter collaborative consortium, which set up a large prospective cohort of nephrotic syndromes) excluding any possibility of studying gut microbiota in this context [5].

The gut microbiota is a complex microbial ecosystem composed with more than 100 billion bacteria, archaea, viruses, parasitoids and fungi which maintain symbiotic interactions with the host. As in many chronic non-communicable diseases, the gut microbiota is emerging as a “key organ” in progression and complication in acute and chronic kidney disease (CKD) [6]. Several studies have clearly demonstrated that composition of gut microbiota is altered (a phenomenon called dysbiosis) in CKD patients [7]. Similar findings have been observed in children with CKD where dysbiosis mediates the proinflammatory immune phenotype [8], leading to the release and the accumulation of some molecules (called gut-derived uremic toxins) produced by gut bacteria and that have pathogenic effects. Outside of this axis, the gut microbiota constantly secretes molecules that are key for the development and maintenance of a homeostatic immune system. As a consequence, the understanding of the role of gut microbiota in immune-mediated kidney diseases is a very hot topic.

In the current issue of Nephrol Dialysis Transplantation, Wang et al. [9] focused on the link between gut microbiota and INS, and tried to determine whether a specific microbiota pattern could predict relapse in children with INS. They reported a decreased abundance of butyrate [a prototypical short chain fatty acid (SCFA)]-producing bacteria before treatment with steroids in early (before 1 year) relapsing patients. They supposed that the reduction of the fecal concentration of butyrate could explain, at least partially, the modification of the differentiation and induction of regulatory T cells (Treg) (a particular subset of CD4+ T cells associated with regulatory functions, previously described by others as being reduced in patients with INS). In good agreement, Tsuji et al. [10] recently reported that, in eight children with relapsing INS before steroids exposure, the proportions of fecal SCFA production and butyric acid–producing bacteria were lower than in non-relapsing children. In adult patients with INS before specific treatment, again the composition of gut microbiota was found to be altered compared with healthy patients, with a marked reduction of SCFA-producing bacteria [11, 12]. Wang et al. [9] also described a change in the ratio of anti- and
pro-inflammatory bacteria which could also participate in the relapse of this autoimmune disease. However, in this study [9] the signature of a reduction of butyrate-producing bacteria in feces in children with INS relapse was never observed in children after steroid exposure. Previously, it was demonstrated that 4 weeks after the initial therapy with steroids, the proportion of fecal SCFA-producing bacteria in 20 children with INS was increased [13]. In 17 children with INS, an increase of Treg cells and butyric acid–producing bacteria were observed when remission had been achieved [14]. An important point must, however, be underlined: in this study [9], samples from the same INS children at onset and after remission were not collected, and this could account for the discrepancies. Overall, if all these preliminary observations seem to be consistent and indicate a role of fecal butyrate in the onset and/or relapse of INS, they nevertheless need to be interpreted carefully given the small number of patients, the heterogeneity of the population and the lack of renal histopathological classification in most cases. Furthermore, the modification of Treg cells in all these studies must be carefully interpreted since several confounding factors (such as the immunosuppression, infection) can influence this lymphocyte populations. This observational study [9] raises many interrogations regarding the possible link between gut microbiota and glomerular diseases.

First, challenges in INS-specific biomarker discovery include determining the causality of observed changes, understanding their functional redundancy in INS mechanisms, and the geographic and ethnic heterogeneity of gut microbiota. Indeed, in this study and in all those cited previously, all participants were from Asian origin which weakens the generalizability of these findings to other populations. The use of fecal transplantation to demonstrate the causal link between gut microbiota composition and phenotype is limited. Mechanically, butyrate modulates the host’s biological responses through several mechanisms. Butyrate has been proven to stimulate the differentiation of Treg cells in vitro as well as in vivo [15]. It has been linked to stimulation of mucus synthesis and improvement of tight junctions which reduce gut permeability and prevent the abnormal passage of luminal antigens and bacterial products into the lamina propria. These properties may explain the emerging key role of SCFA in kidney disease. In CKD, several studies confirm a decreased abundance of SCFA-producing gut bacteria in parallel with a reduced fecal concentration of butyric acid [16, 17]. This reduction was independently associated with an increased mortality in hemodialysis patients [18]. To clearly demonstrate the causality, one strategy could be to directly administrate a butyrate-producing bacterium, as a probiotic, or to selectively stimulate growth of these bacteria via the diet (using a high-fiber diet or prebiotics) and look for a reduction of the rate of INS relapse. In other kidney diseases, several experimental data confirm the positive impact of these strategies (e.g., improved tolerance in kidney transplant model [19], reduced renal fibrosis [20] and increased T-cell commitment toward Treg cells [21] in CKD mice). In an open randomized study, children in remission from primary INS have been treated or not with 3 g per day of Clostridium butyricum at the end of the 8-week steroid administration period. If this probiotic seems to be efficient in increasing the relative abundance of butyrate-producing bacteria and number of blood Treg cells, the reduction in relapses observed in this study needs to be consolidated in view of the very small number of patients involved (n = 10) [22]. We are still far from being able to state with certainty that decreased fecal butyrate is a biomarker and/or a therapeutic target to prevent INS relapses. Large-scale multicenter prospective studies will need to be performed to confirm the cause-and-effect relationship of these changes in the microbiota of INS patients.

Secondly, the generation of these data on intestinal microbiota are not fully in accordance with the recent guidelines for human gut microbiome studies [7]. Lack of care in sample collection, choice of controls and characterization of sick subjects may be sources of significant bias. For example, in this cohort, very little information is given regarding geographical location (for example, urban versus non-urban locations), BMI and diet habits known to influence the gut microbiota. The baseline characteristics of healthy controls are lacking. Scarce information is provided about the practical organization of sample collection and storage. Indeed, it is of prime importance that fecal collection be immediately conserved at −20°C until transfer to the laboratory or the hospital facility, where they should be stored at −80°C until further processing. As suggested recently by Randall et al. [23], in rodent studies there is no current evidence that CKD causes reproducible patterns of dysbiosis, and they suggest meta-analysis of repository data as a way of identifying broad themes that transcend experimental variation. A similar approach is necessary for clinical studies in CKD.

Third, Wang et al. [9], like the prior studies, characterized the gut microbiota using only the cost-effective 16S ribosomal RNA gene sequencing. However, a better taxonomic resolution and/or a functional profile would be needed to better understand the complexity of the microbiota, using a shotgun metagenomic sequencing approach for future studies. These methods, however, involve deep sequencing coupled with high-throughput bioinformatics. These techniques are expensive and time-consuming, and require significant technical expertise to design, run and analyze. The continuous improvement of metagenomic workflows, however, allows us to hope that this technique could be used routinely in future [24].

Finally, the authors focused on SCFA and particularly butyrate, but many other gut-derived microbiota products may modulate or interfere with the immune system. For example, tryptophan-derivate molecules such as indole are potent ligands of the aryl hydrocarbon receptor (AhR) which is known as a regulator of Treg immunity [25]. More generally, many uremic toxins are described in CKD as influencing the immune system, either regulatory or inflammatory [26]. Also, the gut microbiota composition can inactivate, activate and change the potency and bioavailability of drugs through direct alteration of enzyme activities. The most spectacular example of the gut microbiota influencing therapeutic responses is probably immunotherapy. Indeed, the gut microbiota composition deeply influences clinical response to anti-PD-1 therapy in melanoma patients [27]. In this study [9], the authors confirm that steroids can change the gut microbiota composition, but the clinical significance of this observation and the impact of other immunosuppressives drugs in INS must be fully investigated.

In conclusion, the Wang et al. study [9] encourages us to continue to explore the role of gut microbiota in glomerular diseases in the future. Although we are still far from being able to integrate the composition of the microbiota in the management of INS, we expect that a better understanding of the relationships between gut microbiota, immune system and/or renal function could enrich our guidelines for a personal management for prevention, treatment and adaptation of therapeutic choices of immunosuppressants (Fig. 1). There are still many obstacles that will probably be overcome in the next few years, amongst others by technological advances in multi-omics analysis such as the...
integration of the wide inter-individual variability, the quality of the collection/analysis of gut microbiota, and the elaboration of large and accurately phenotyped cohorts.

**CONFLICT OF INTEREST STATEMENT**

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