Effect of Combined Gluten-Free, Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome: An Open Pilot Trial

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Juliette Leon[1], María José Pérez-Sáez[1,2], Audrey Uffing[1], Naoka Murakami[1], Andreia Watanabe[3], Pamela Cureton[4], Victoria Kenyon[4], Leigh Keating[5], Karen Yee[5], Carla Aline Fernandes Satiro[6], Bryant Yu[1], Joseph V. Bonventre[1], Alessio Fasano[4] and Leonardo V. Riella[1]

[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; [2]Servicio de Nefrología, Hospital del Mar, Barcelona, Spain; [3]Department of Pediatrics, Pediatric Nephrology Unit, Instituto da Criança, Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil; [4]Center for Celiac Research and Treatment, Massachusetts General Hospital for Children, Harvard Medical School, Boston, Massachusetts, USA; [5]Center for Clinical Investigation, Brigham and Women's Hospital, Boston, Massachusetts, USA; and [6]Division of Nutrition, Instituto da Criança, Hospital das Clínicas, University of Sao Paulo Medical School, São Paulo, Brazil

Introduction: Steroid-resistant nephrotic syndrome (SRNS) affects both children and adults and has a high rate of progression to end-stage renal disease. Although a subset of patients have well-characterized genetic mutation(s), in the majority of cases, the etiology is unknown. Over the past 50 years, a number of case reports have suggested the potential impact of dietary changes in controlling primary nephrotic syndrome, especially gluten and dairy restrictions.

Methods: We have designed a prospective, open-label, nonrandomized, pilot clinical trial, to study the effect of a gluten-free and dairy-free (GF/DF) diet in children with SRNS. The study will be organized as a 4-week summer camp to implement a GF/DF diet in a tightly controlled and monitored setting. Blood, urine, and stool samples will be collected at different time points during the study.

Results: The primary end point is a reduction of more than 50% in the urine protein:creatinine ratio. The secondary end points include changes in urine protein, kidney function, and serum albumin, as well as effects in immune activation, kidney injury biomarkers, and gut microbiome composition and function (metagenomic/metatranscriptomic).

Conclusion: This study will advance the field by testing the effect of dietary changes in patients with SRNS in a highly controlled camp environment. In addition, we hope the results will help to identify a responder profile that may guide the design of a larger trial for further investigation.

I diopathic nephrotic syndrome (INS) is the most common type of nephrotic syndrome in children, presenting clinically with massive proteinuria, hypoalbuminemia, hyperlipidemia, and edema. Despite recent advances in identifying genetic mutations in a subset of these patients,[1] in the large majority of cases of nephrotic syndrome, the etiology is unknown.[2] The underlying lesion leading to the severe proteinuria is a defect in the glomerular filter barrier, with diffuse effacement of podocyte foot processes on electron microscopy and without glomerular deposits or inflammatory lesions. Two different histopathological patterns may be identified by light microscopy: minimal change disease (MCD),[3] without optical changes in glomeruli, and primary focal segmental glomerulosclerosis (FSGS),[4] with scar lesions visible inside glomeruli.

The clinical course and prognosis of INS is widely dependent on the response to initial treatment course with steroids, which itself is closely related to the distinct histological pattern. Although 80% to 90% of children with INS achieve complete remission with the initial course of steroid therapy and are classified as having steroid-sensitive nephrotic syndrome (SSNS),[5] about 10% to 20% present with a lack of response to steroids (steroid-resistant nephrotic syndrome [SRNS]), or experience frequent relapses after withdrawal of steroids,

Correspondence: Leonardo V. Riella, Transplantation Research Center, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, Massachusetts 02115, USA. E-mail: lriella@bwh.harvard.edu

resulting in steroid-dependent nephrotic syndrome (SDNS).[6] SRNS and SDNS are predominantly associated with an FSGS pattern in more than 60% of cases, whereas SSNS is associated with an MCD pattern. Therefore, common practice is to only perform a kidney biopsy to determine the exact pathological findings after steroid treatment for 4 to 8 weeks without clinical response.

It has been proposed that MCD and FSGS are different histological patterns of the same disease, representing a spectrum, with MCD dominating the initial presentation and the continuous podocyte injury leading to FSGS, although this remains controversial.[7] In a subset of SRNS, causative genetic mutations have been discovered.[1] When no genetic mutation can be found, it is hypothesized that toxic circulating factor(s) causes podocyte injury and consequently increases glomerular permeability. This could also explain the high rate of recurrence of the disease after transplantation.[6,8] The exact nature of this circulating factor(s) is still unknown, but it is thought that the immune system could be a major culprit.[9–13]

Without response to steroids and therefore persistent proteinuria, SRNS poses an enormous therapeutic challenge. In addition to the inevitable progression to end-stage renal disease, other complications such as malnutrition, infection, and thrombotic events often occur.[5,6] Children with SRNS are usually started on a variety of immunosuppressant drugs such as calcineurin inhibitors, mycophenolic acid, and/or, in cases with difficult courses, alkylating agents and rituximab, with variable success rates and significant side effects.[14,15] Despite the use of newer immunosuppressive agents, the response rate to therapy remains low.[14,15] To find novel therapeutic strategies for SRNS, it is critical to investigate potential etiologies and biomarkers for this syndrome. Over the past 50 years, a number of case reports have suggested the potential impact of dietary changes in controlling INS, likely related to food sensitivity.[16,17] In particular, gluten and dairy restrictions have been associated with a significant decrease in proteinuria, both in SSNS/SDNS and in SRNS.[18–21] In 1977, Sandberg et al.[20] studied 6 children with INS and demonstrated significant reduction in proteinuria (<0.5 g/d) after the removal of cow's milk from the diet, with exacerbation of proteinuria once patients were rechallenged with cow's milk. In 1989, Laurent et al.[18] investigated the relation between INS and food sensitivities in pediatric and adult INS patients (age range 7–72 years). They investigated a broader collection of foods, including cow's milk, egg, chicken, beef, pork, and gluten. Among 26 participants, 6 responded to dietary interventions and achieved complete remission (CR): 2 patients after gluten avoidance; 3 after removal of cow's milk; and 1 after

removal of beef, pork, and egg white. Milk sensitivity was also reported in 6 of 17 children with INS, whose proteinuria improved after milk exclusion from the diet.[22] Lagrue et al.[17] implemented an oligoantigenic diet (which included removal of milk and gluten) in 42 patients with difficult-to-manage INS. They found that 13 of these patients achieved >50% reduction in proteinuria and 5 achieved CR. In most of the patients who responded, the time onset to response was within 1 week, and INS recurred immediately when the restricted diet was stopped. More recently, Lemley et al.[19] reported a case series of 8 children (2–14 years of age) with difficult-to-manage INS who were started on gluten-free diet. All patients experienced a significant reduction in the relapse rate, enabling lower doses or withdrawal of steroids or immunosuppressive drugs. In this pilot study, we decided to focus on SRNS, as it is the most orphan entity in terms of therapeutic approaches and the greatest challenge among all INS varieties. In addition, the knowledge of successful responses to gluten/dairy removal in cases of SRNS, including patients of our coinvestigators, encouraged us to target this specific population first.

The exact mechanism by which dietary interventions can reduce proteinuria is unknown, but several hypotheses have been proposed (Figure 1). Different from food allergy that is mediated by an IgE response, food sensitivity is linked to immune cellular dysfunction and is difficult to diagnose, as no circulating antibodies or skin tests have shown a reliable correlation.[17–19,21,23,24] Exposure to sensitive foods may trigger the release of inflammatory factors/cytokines that could directly damage the podocytes (Figure 2).[19]
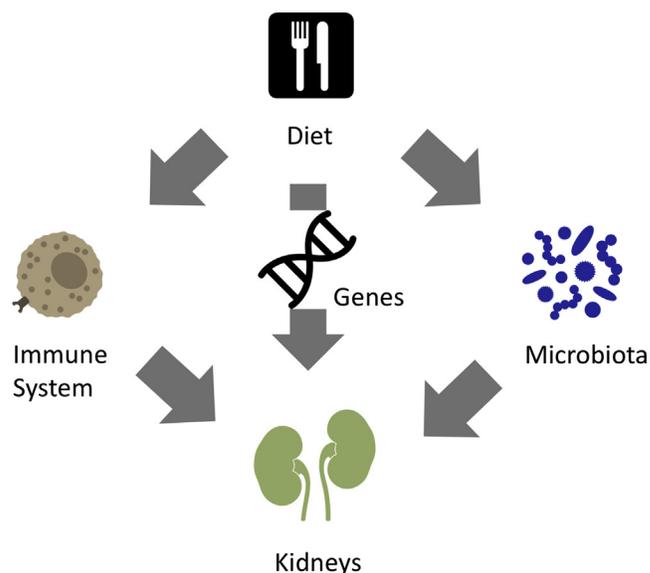

Figure 1. Illustration of the hypothesis behind a dietary intervention influencing the immune system activation, microbiota, and kidneys.

This effect could be mediated in part by contributions from the microbiota.[25] It has also been hypothesized that gluten-sensitive patients experience an increase in the secretion of a molecule called zonulin, a major modulator of intercellular tight junctions.[26] This molecule might then open tight junctions in the gut epithelium, and could therefore increase permeability of gut epithelium to potentially toxic proteins produced by the microbiota.[24,27] Finally, circulating zonulin might potentially have a direct effect on podocytes, which has been shown in preliminary data in mouse podocyte cultures (Schramm K, Faul C, Zonulin, a circulating factor that regulates podocyte function and glomerular permeability. Abstract Supplement for the American Society of Nephrology [ASN], November 2014, Abstract SA-PO435). Overall, these reports support the rationale of our study hypothesizing that dietary intervention avoiding gluten and dairy, alone or in addition to immunosuppressive therapy, might target specific pathogenic pathways involved in podocyte injury.

Based on the limited risks of dietary change compared to aggressive immunosuppression used in this cohort and the lack of reliable clinical tests to diagnose food sensitivity other than food avoidance, we have designed a prospective, open-label, nonrandomized pilot clinical trial to study the effect of a gluten-free and dairy-free (GF/DF) diet in children with SRNS (Figure 3). To avoid dietary deviations, the study is organized as a 4-week summer camp, in which professional chefs provide all meals. Moreover, the camp aims to create a friendly and educational environment in which participants can meet other children and families with the same medical condition. Finally, this study will allow us to identify patients who might respond to this dietary intervention, to address a responder profile and, afterward, to improve the screening of those who could have a potential response to diet.

METHODS

This is a pilot dietary intervention study, in which a strict GF/DF diet is investigated during 4 weeks at a summer camp in Orlando, Florida. Patients are not randomized, and there is no control group.

Study Participants, Screening (Day −70 Through Day 0), and Enrollment

Patients 2 to 21 years of age with SRNS, biopsy-proven primary FSGS or MCD are eligible to participate in the study. Inclusion and exclusion criteria are presented in Table 1.[28]

We anticipated an enrollment of 20 to 30 subjects worldwide, from various recruitment sites including academic hospitals, research centers, nephrology physicians, community nephrology clinics, and directly via patient associations and families. Recruitment for clinical trials is a challenge, especially due to the rare nature of the disease. To achieve adequate enrollment, we delivered information on the trial using different means of communication, such as via physician research groups, patient advocacy groups (e.g., NephCure Kidney International), social media (e.g., Facebook), and our own study website (www.thegeniestudy.com).
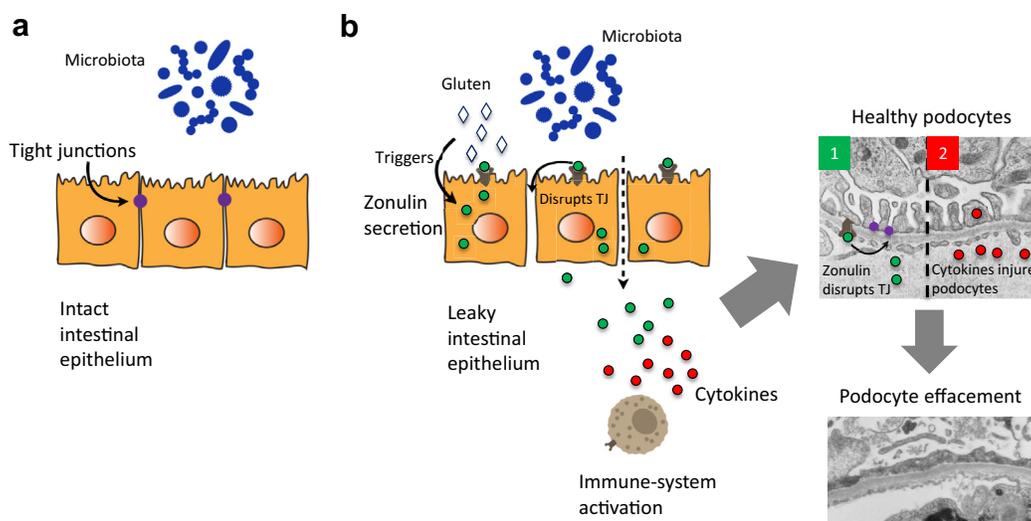

Figure 2. Illustration of the potential mechanisms involved in gluten-dependent podocyte injury. (a) Normal intestinal epithelium with intact tight junctions (TJ) and intraluminal microbiota. (b) Intestinal epithelium from patients with gluten sensitivity. Upon gluten exposure, intestinal cells increase zonulin secretion, a protein that can disrupt intercellular tight junctions. (1) Elevated circulating zonulin could then have a direct effect on podocytes by disrupting their tight junctions and consequently, leading to slit diaphragm malfunction and proteinuria. (2) Alternatively, disrupted tight junctions in intestinal epithelial cells may increase the permeability of the gut, which allows toxins produced by the microbiota to enter the circulation and to activate the immune system. Subsequent cytokine production by immune cells may cause injury to podocytes in the kidney.
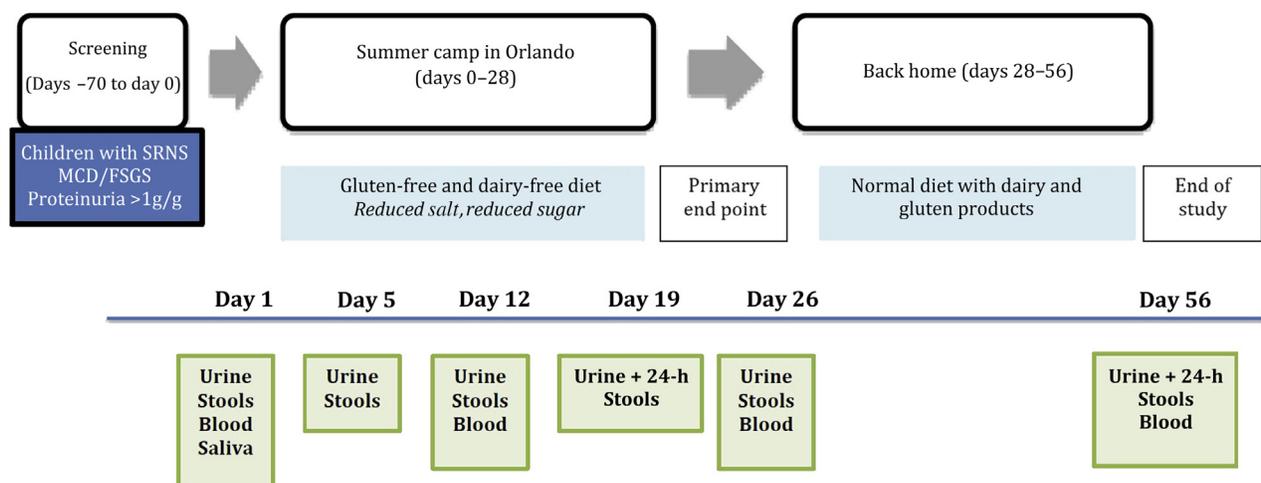
Figure 3. Sample collection and organization at Gluten-Free/Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome (GENIE) camp for children with steroid-resistant nephrotic syndrome (SRNS). FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease.

Patients who expressed their interest in the study were interviewed by the study coordinators to review inclusion and exclusion criteria. Further medical information was obtained from the treating physician, including a biopsy report. Written informed consent was provided to the study participants before any interventions during the screening period. Finally, 17 subjects were recruited, who signed the informed consent form.

The Gluten-Free/Dairy-Free Diet In Children With Steroid-Resistant Nephrotic Syndrome (GENIE) study obtained Brigham and Women's Hospital institutional review board approval in a timely manner (2017P000615/PHS).

Study Design and Setting

Patients participate in the study for a total of 8 weeks (from the third week in July until the third week in September 2017), including 4 weeks in the camp. Figure 3 represents the study design, divided into 2 parts after the screening period.

Before arriving at the camp, subjects complete a food record including 2 weekdays and 1 weekend day to assess intake before the intervention.

Summer Camp in Orlando (Day 0 Through Day 28)

During the first 28 days, the GF/DF diet is provided for all participants and their attending parent/guardian at the camp, as the latter stays on-site during the whole camp period. On day 0, information is given about the camp, rules, activities, sample collection, and the diet. On day 1, all participants undergo an extensive clinical examination, followed by baseline sample collection (blood, urine, stool, and saliva). Afterward, the GF/DF diet is initiated.

Sample Collection and Processing

Samples are collected at different time points during the study, as detailed in Figure 3; stool and first morning urine are collected weekly, and blood is collected at 3 time points (days 0, 12, and 26).

Table 1. GENIE study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Male or female, 2–21 yr of age	1. Kidney disease other than FSGS or MCD
2. Willing and able to provide written informed consent signed by patient if 21 yr of age, or parent/guardian willing in any other cases, to comply with dietary changes	2. Patient with known gluten or milk allergy, proved by immunological tests
3. SRNS defined as persistent nephrotic-range proteinuria after at least 8 wk of steroid therapy (starting with 60 mg/m^2 per day or 2 mg/kg per day to a maximum of 60 mg/d for 4−6 wk and following as alternate-day medication)*	3. Patient with known homozygous podocyte genetic mutation
4. Biopsy-proven FSGS or MCD	
5. eGFR > 50 ml/min defined by bedside Schwartz formula (GFR = [height in cm] × 0.413/serum creatinine mg/dl)	
6. UPCR ≥ 1 g/g at screening with 2 different samples collected within the 3 months before camp	
7. Concomitant use of ACEi/ARB at stable doses for at least 2 wk before camp	
8. No changes in immunosuppression for the previous 2 mo	

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; GENIE, Gluten-Free/Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome; GFR, glomerular filtration rate; MCD, minimal change disease; SRNS, steroid-resistant nephrotic syndrome; UPCR, urine protein:creatinine ratio.
Based on KDIGO Clinical Practice Guideline for Glomerulonephritis. *Kidney Int Suppl* 2012;2:143–153.[28]

On the day of sample collection, all samples are shipped overnight to Boston, Massachusetts, on ice, except whole blood in ethylenediaminetetraacetic acid, which is shipped at room temperature (Figure 4). Samples are processed upon arrival: serum is aliquoted and stored at −80°C for future testing. A small amount (100 μl per patient) of whole blood in ethylenediaminetetraacetic acid is used for fresh flow cytometry analyses (Figure 4).

The Intervention: The Diet

The diet consists of GF/DF and no added salt or low salt for all subjects, family, and staff. Meal plans for the first week are developed before the camp, and are assessed for balance and compliance with the United States Department of Agriculture (USDA) Food and Nutrition Service My Plate guidelines,[29] adapted to each age group and for dairy exclusion. During the camp, the dieticians are working closely with the chefs to create nutritionally balanced meals and to ensure healthy food options, as well as to adapt the diet to the children's preferences. Table 2 shows a menu example.

For the compliance of the GF/DF diet, the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and Children's Digestive Health and Nutrition Foundation (CDHNF)[30] guidelines are followed, and all food and drink items containing gluten are avoided, for example, wheat, barley, rye, triticale, and oat without a ''gluten-free'' label. Processed food only with the label ''gluten-free'' is allowed, although the diet is based mainly on naturally gluten-free organic food. All of the kitchen utensils are new to prevent cross-contamination. Furthermore, 1 kitchen in the camp is responsible for the storage, preparation, and distribution of all foods, including meals and snacks during the camp and
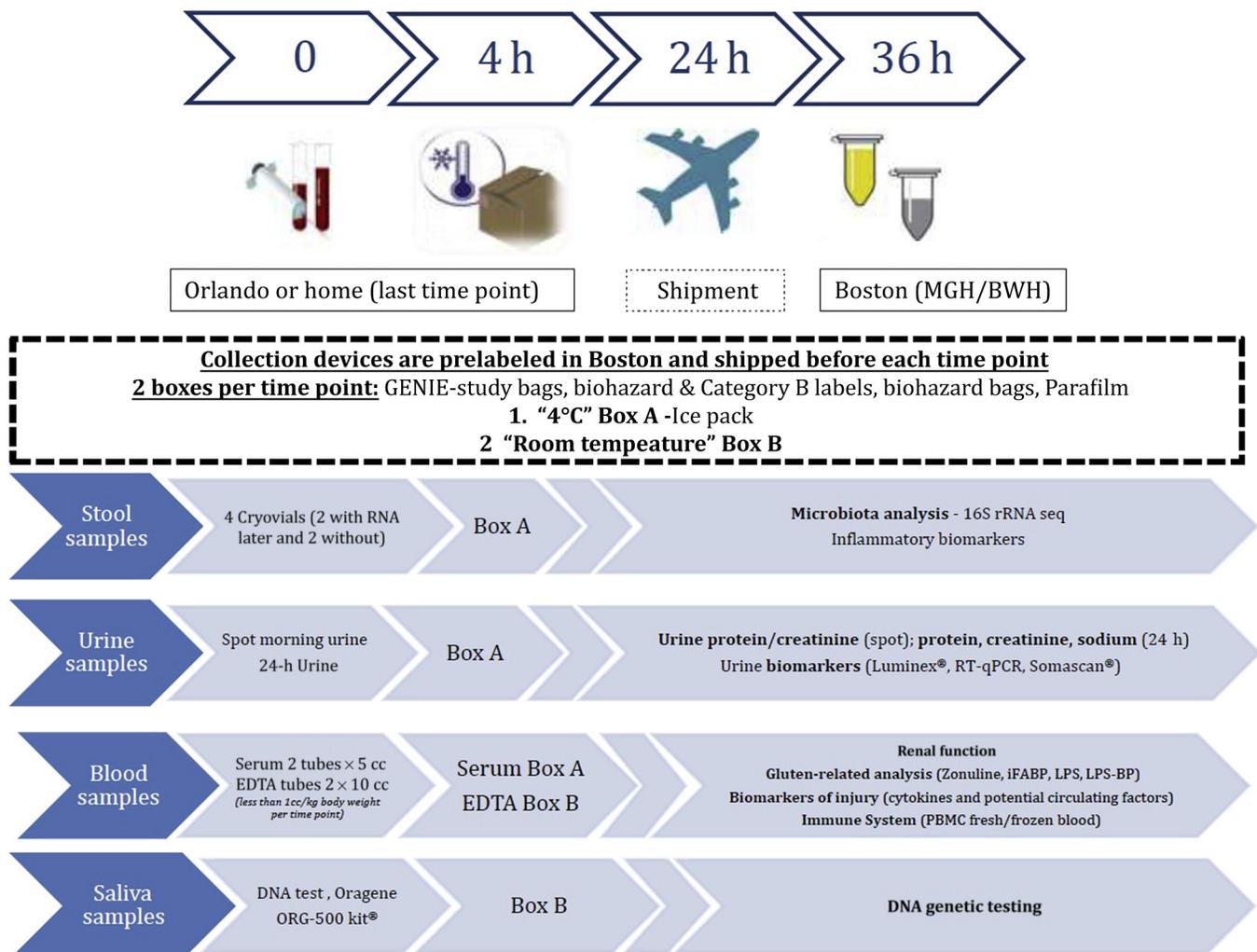

Figure 4. Sample processing and tests planned for Gluten-Free/Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome (GENIE). BP, binding protein; BWH, Brigham and Women's hospital; EDTA, ethylenediaminetetraacetic acid; iFABP, intestinal fatty acid−binding protein; LPS, lipopolysaccharide; MGH, Massachusetts General Hospital; PBMC, peripheral blood mononuclear cell; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

Table 2. Example of meals/activity during 1 day at GENIE camp

Daytime	Activities	Menu
Morning	Circus activities // Singing // Yoga Aquatic park activities	Breakfast (8–9 AM) Almond milk (coconut powder / coffee) Gluten-free bread, cereal, or waffles with home-made strawberry jam Scrambled eggs Fruit (mango or berries) Snack at 11 AM: fruit
Afternoon	Board games, video, arts Cooking workshop // Create a puppet	Lunch (12–1 PM) Salmon with orange sauce Side: salad with rice, lentils, lettuce, tomato and green collard Desert: Papaya cream Snack at 3:30 PM: muffin or strawberry-banana smoothie
Evening	Trip to Walt Disney World once a week or Quiet activities (board games, reading) or Special theme evening every Saturday	Dinner (6–7 PM) Pea soup Grilled corn with roasted chicken Salad of mixed greens with mango sauce Desert: dairy-free ice cream Snack at 9 PM: herbal tea, cookies, and fruit

GENIE, Gluten-Free/Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome.

snacks that are taken with participants during outside camp day trips.

Regarding a "no added salt/low-salt" diet, children start with no added salt diet unless by specific previous medical recommendation. During the camp, and depending on symptoms (such as low blood pressure), a maximum of 2000 mg of salt (800 mg of sodium) per day is allowed. No fluid restriction is mandatory during the camp. However, hydration state is checked as frequently as needed. Therefore, the salt intake and water intake might change during the camp period, at the physician's discretion.

Children receive daily calcium supplementation, based on the dietary reference intakes developed by the USDA, for each age frame, ranging between 700 mg and 1300 mg daily.

Children are asked to use stickers to fill out a food-track form after every meal, to track food intake and to ensure nutrition standards for the children (Supplementary Figure S1). The dieticians review food logs every week. Educational and learning activities focused on the GF/DF diet are implemented during the camp, for example, lectures about food and GF/DF diet, cooking workshops for children and parents given by the chefs and dieticians, and visits to organic food stores to learn how to select and to purchase healthy food.

Clinical Follow-up, Medical Supervision, and Treatment
At least 1 research physician is present on site during the whole camp period, to medically observe the enrolled children. A clinical examination is performed once a week with the focus on tolerance and acceptance of the

GF/DF diet. The participants are allowed to continue the concomitant medication regimen upon enrollment, such as renin–angiotensin system blockade agents, aldosterone blockers, diuretics, and immunosuppressive drugs. As we are introducing an intervention with the diet, adjustments in diuretics or antihypertensive agent dose are allowed if necessary. In case of discontinuation from the study, the reason(s) are carefully documented, especially if adverse events occur.

Back Home Without the GF/DF Diet (Day 28 Through Day 56)
After the 4-week camp, participants return home to their usual dietary behavior for 1 month, although they are encouraged to maintain a low-salt diet and to avoid processed foods. A food diary should be completed 2 weekdays and 1 weekend day during the month. On the last day of the study (day 56), the samples are collected locally with the material provided at the end of camp (day 28) (Figure 3).

Potential Risks
No life-threatening risk is related to the GF/DF diet in the literature, and the main reported adverse event is constipation, which is usually related to inadequate consumption of fiber and not directly related to gluten avoidance. On the other hand, restricting dairy products may lead to a decreased calcium intake, so supplemental calcium is provided as described above. In addition, the proposed diet contains large quantities of fruits and vegetables and therefore a higher content of potassium, which might potentially lead to a risk of hyperkalemia. Although we exclude patients with significant kidney dysfunction (estimated glomerular filtration rate < 60 ml/min), who are at higher risk for hyperkalemia, we plan to monitor potassium twice during the camp, because some patients are on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, which could raise serum potassium levels. A potassium restriction diet will be introduced if hyperkalemia is observed. After returning home from the camp, children will be exposed to gluten and dairy products again, which could lead to several symptoms, such as gastrointestinal bloating or diarrhea in gluten-sensitive patients, and cramps, bloating, or diarrhea in dairy-sensitive patients, without life-threatening symptoms described. In case we discover gluten or dairy sensitivity during the study in any of the participants who might encounter symptoms during re-exposure, we encourage them to contact the study team and their own physician to assess their symptoms and to provide additional help. To ensure that symptoms are captured during the study, gastrointestinal symptoms are recorded before

and after camp, to identify potential subjects at risk for gluten sensitivity.

Whole-exome sequencing may entail the risk of negative emotional, social, and/or financial consequences of test results. Participants and/or their guardian are explained these risks upon providing consent. Genetic test results will not be placed in medical records, and information on (podocyte-specific) mutations will be released to participants only if they have agreed to receive these results.

Camp Structure and Organization

Because of kitchen limitations with our restricted diet and risks of cross-contamination, we are organizing a unique camp inside a summer resort in Orlando, Florida. Three houses are part of the camp, with 1 house dedicated to meal preparation and dining, and the research staff housing. The daily activities for the participants are coordinated by an activity team, consisting of 5 animators, who are present at the camp 6 days a week from 9 AM until 5 PM. Activities include local activities in the resort and weekly trips to entertainment parks in the area. Campers are allowed to choose different activity intensities based on their health conditions, as is described in Table 2.

Outcomes and Assessments

The main objective of the study is to determine whether a GF/DF diet decreases proteinuria in the patients with SRNS. Therefore, the primary end point is a reduction of more than 50% in urine protein:creatinine ratio (UPCR) from day 0 to the end of the GF/DF diet (day 28). Secondary end points include the following: (i) change in UPCR between day 0 and day 28, as well as the proportion of patients achieving complete remission (UPCR $\leq$ 0.5 g/g); (ii) changes in serum albumin; (iii) creatinine and estimated glomerular filtration rate (eGFR) defined by Bedside Schwartz formula (GFR = [height in cm] $\times$ 0.413/serum creatinine mg/dl); (iv) changes in blood pressure; and (v) changes in weight.

In addition, we will analyze biomarkers of kidney injury by SOMAscan (Somalogic, Inc., Boulder, CO) and Luminex (Luminex Corporation, Austin, TX); serum levels of zonulin, lipopolysaccharide, lipopolysaccharide-binding protein, and intestinal fatty acid–binding protein by enzyme-linked immunosorbent assay; systemic, gut, and urine inflammatory biomarkers (such as cytokines) by Luminex; immunophenotyping of peripheral blood assessed by flow cytometry; autoimmune antibodies assessed by Luminex; and changes in gut microbiota composition (microbiome profiles will be determined using 16S rRNA sequencing and then processed for taxonomic and imputed functional composition).

Furthermore, patients will be genetically characterized to find potential genetic causes of SRNS. Genetic testing

(whole-exome sequencing, Illumina HiSeq [Illumina Inc., San Diego, CA], 180 Gb per sample) will be performed using a saliva sample, obtained on day 1.

The safety and tolerability of the diet is assessed with the documentation of physical parameters. Especially, medications required for controlling fluid volume and any dosage changes are documented. Even though the environment and diet of the study will be thoroughly controlled, the compliance regarding the GF diet is assessed once during the camp in a urine random sample, by a gluten dipstick (IVYDAL, Biomedal S.I., Sevilla, Spain) that is able to detect the presence of gluten in urine.[31] After reintroducing gluten at home, compliance is assessed again in a urine sample.

Data Management and Safety

Before, during, and after the camp, all patient data are recorded in an electronic database (Redcap), including information that was obtained for screening purposes, such as biopsy reports and patient records. To ensure confidentiality of data, participants are assigned a study code, and the database is protected with a password. Only study staff has access to the database and personal identifiers connected to the study codes. Variables collected include the following: demographic data, family history, past medical history, kidney disease history, biopsy findings, therapeutic management (medications used, duration, response), genetic testing, birth history and extrarenal manifestations, allergy history, immunizations, laboratory findings before camp (creatinine, albumin, UPCR, cholesterol, Ig levels, complement levels, presence of hematuria), current treatment, clinical examinations on days 1 and 28, and all follow-up data during the study period (days 0–57) regarding laboratory results, clinical visits, and adverse events, if any.

Samples and health information of participants may be shared with outside scientists for further testing. If this is the case, samples and health information will be deidentified following Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations.

Sample Size and Statistical Analyses

This is a pilot trial to gain further insight into the potential effect of a GF/DF diet in children with SRNS, and thus is not powered to obtain a definitive answer. The goal is to estimate the size and variability of the GF/DF diet effect so that the sample size required for a future study can be properly estimated. We planned to recruit 20 to 30 patients, based on the feasibility of the recruitment and housing as well as prior literature on recommended sample sizes in pilot trials.[32,33] We finally recruited 17 subjects for the study.

Statistical analyses will be performed using Stata13 software (StataCorp, College Station, TX). The tests will be 2-sided, with a type I error set at α = 0.05. The

baseline characteristics will be presented as mean ($\pm$ SD) or median (interquartile range) values according to the statistical distribution for continuous data (assumption of normality assessed using the Shapiro–Wilk test) and the number of patients and associated percentages for categorical parameters. Comparisons of patient characteristics between independent groups will be conducted using the χ^2 or Fisher exact tests for categorical variables and the Student t test or Mann–Whitney U test for quantitative parameters (homoscedasticity verified using the Fisher–Snedecor test).

DISCUSSION

In this study, we hypothesize that a GF/DF diet introduced after the diagnosis of SRNS might help to reverse nephrotic-range proteinuria.

Currently, we are facing a therapeutic impasse in SRNS, which causes a clinical need to develop safe, well-tolerated and nephron-protective therapies, especially in children. There is no US Food and Drug Administration–approved therapy for SRNS, and immunosuppressant drugs that are used as second-line treatment are undesirable because of toxic side effects and limited effectiveness. Because dietary interventions such as the one proposed in this study have shown promising results and have minimal risks, we hope that this pilot trial will benefit future patients by providing further insight into the potential effect of a GF/DF diet on outcomes in children with SRNS.

In the preceding reports of dietary interventions in INS, removal of gluten and/or dairy was only effective as treatment—a significant add-on effect on proteinuria reduction—in specific cases, and therefore it is likely that not all participants will benefit from study participation. Moreover, although the case reports are intriguing and promising, the variety of methods of implementation of the elimination diet in terms of stringency, control, duration, and readouts leave ample opportunity for a better design approach to identify who, among children affected by INS, can benefit from this therapeutic approach.

Therefore, one of our important aims is to characterize patients who respond to the intervention, to analyze genetic predispositions and potential biomarkers in blood, urine, and feces, to stratify the patient population to identify the subgroup in which the implementation of a GF/DF elimination diet can have therapeutic efficacy.

The study is designed as a summer camp for several reasons. A summer camp is a common activity during summer vacation for many children in the United States. However, for children with chronic diseases, attendance at a summer camp is often complicated or not possible because of their medical condition. As a result, special summer camps for chronically ill pediatric patients have emerged since the 1990s, especially in asthma, diabetes, and obesity.[34–36] In nephrology, there is limited published work on summer camps for children on dialysis,[37] and, to our knowledge, so far no camp has been designed specifically for the implementation of new therapies in kidney patients. The camp provides an opportunity for these children to experience a summer camp, to increase their independence from their parents, and to spend time with other children who have the same disease,[38] while also providing appropriate medical care and education.[39] Epstein *et al*. described how 4 fields of health-related quality of life could positively be affected by summer camps for children: physical, cognitive, social, and psychological quality of life.[40] As kidney diseases are usually not very frequent during childhood, the summer camp offers a special added value in these children, as they are able to meet and share experiences with other children with the same conditions.

On the other hand, the summer camp design is specifically valuable here, because it offers the opportunity of introducing and complying with a GF/DF diet in the most appropriate and accurate way: chefs will cook all meals and snacks for all participants, and everyone at the camp will follow the diet. This eliminates difficulties that could occur at home, such as accidental intake of gluten/dairy due to a lack of dietary knowledge, temptations to eat restricted foods, and the need to remove all gluten-containing ingredients at home. Finally, this design ensures an easy and standardized means of sample collection, as conditions for all participants and samples are equal, and provides the opportunity for close medical supervision and detailed follow-up.

It is important to recognize the potential limitations of the Gluten-Free/Dairy-Free Diet in Children With Steroid-Resistant Nephrotic Syndrome (GENIE) trial. First, the study sample size is small, and consequently the cohort might not reflect the diversity of children with SRNS. In addition to that, we have used different tools for patient recruitment, including social media, which may create a selection bias in our recruited patients. In particular, as there was a commitment of 4 weeks in a camp, some families may not have the possibility to be away from work for the trial duration. Second, the duration of intervention might not be long enough. Although most patients with SRNS that responded to a dietary intervention showed a quick reduction in proteinuria in the literature,[17–19] only 4 weeks might be too short a period in which to see a significant decrease in proteinuria. Furthermore, the intervention introduces 2 restrictions at the same time

(gluten and dairy), so we will not be able to conclude whether a possible proteinuria reduction is caused by which change. Similarly, the diet will be low sodium, which could also affect the proteinuria results. Finally, there is not a specific control group, but each patient will be their own control when they get back into normal diet between days 28 and 56.

In conclusion, we present the first clinical trial designed to evaluate the impact of a restrictive GF/DF diet on proteinuria in children with SRNS in the course of a summer camp with mechanistic insight into how the diet might affect the kidneys, the immune system, and the microbiota.

DISCLOSURE

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

Figure S1. Example of food track form that children will fill out with stickers after each meal during the camp (for girls 9−13 years old).

Supplementary material is linked to the online version of the paper at www.kireports.org.

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