

# Gluten-Free Diet in Childhood Difficult-to-Treat Nephrotic Syndrome: A Pilot Feasibility Study

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## Keywords

Glomerular disease · Nephrotic syndrome · Proteinuria · Gluten-free diet · Pilot study · Childhood · Difficult-to-treat disease · Zonulin

## Abstract

**Introduction:** Minimal change disease in childhood can follow a frequently relapsing or steroid-dependent course in up to 40% of cases. Second-line immunosuppressive medications that are used to manage these patients are associated with significant adverse effects. There is a need for safer alternative treatments for difficult-to-treat nephrotic syndrome. Therefore, we conducted an open-label feasibility study to assess the safety and efficacy of a gluten-free diet as treatment for pediatric patients with difficult-to-treat nephrotic syndrome. As a second aim, we sought to determine if the plasma zonulin concentration can identify those who are more likely to respond to this intervention. **Methods:** Seventeen patients were placed on a gluten-free diet for 6 months. A positive response was defined as a 50% reduction in the relapse rate compared to the preceding 6 months or

the ability to discontinue 1 immunosuppressive drug. **Results:** Five (29%) participants had a positive response to the dietary intervention. The gluten-free diet was well tolerated with no clinical or laboratory adverse events. Plasma zonulin concentration was elevated in patients who failed to benefit from the gluten-free diet. **Discussion/Conclusion:** A gluten-free diet may be a useful adjunctive intervention for patients with difficult-to-treat nephrotic syndrome that can be implemented prior to resorting to second-line immunosuppressive therapy. Development of the plasma zonulin level as a biomarker to predict efficacy would facilitate rational use of a gluten-free diet in the management of nephrotic syndrome.

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## Introduction

Idiopathic nephrotic syndrome (NS) is the most frequent glomerular disease in childhood [1]. However, it is still a rare pediatric condition with an estimated annual incidence of 1.2–16.9 cases per 100,000 population [1, 2].

Over 80% of cases are attributable to minimal change disease (MCD) which is characterized by a complete remission of proteinuria following a standard course of corticosteroids [2]. Most children with MCD eventually manifest resolution of their disease without any permanent loss of kidney function. Unfortunately, up to 40% of patients with MCD will develop frequently relapsing nephrotic syndrome (FRNS), i.e.,  $\geq 2$  relapses in a 6-month period or  $\geq 4$  relapses in a 12-month period or steroid-dependent NS (SDNS), i.e., relapse on every other day of treatment or within 14 days of discontinuation of corticosteroids [1–3]. Patients with difficult-to-treat disease are at significant risk of serious adverse effects because of prolonged steroid therapy and often require implementation of second-line immunosuppressive therapy to control their disease and alleviate the steroid burden [3]. However, the agents in current use including alkylating agents, antimetabolites, calcineurin inhibitors, and biological agents that deplete B-cells are not uniformly effective and are associated with significant side effects. There is an urgent need to develop novel, potentially personalized approaches to treat patients with FRNS or SDNS.

Over the last 40 years, case reports and patient series have described the impact of food sensitivity and alterations in the diet to control NS in pediatric and adult patients [4–7]. Lagrue et al. [6] detailed their experience with 42 subjects with NS who were evaluated for sensitization to common foods. Use of an oligoantigenic diet, which appears to have excluded gliadin in a subgroup of 13 patients with difficult-to-treat NS, resulted in  $>50\%$  reduction (median decrease 90%) in proteinuria in 9 cases and complete remission in 5 cases. The response was rapid, within a week in most cases, and proteinuria promptly recurred in most responsive patients when the restricted diet was stopped. Long-term steroid-free remissions were achieved by sustained, more specific dietary elimination, including avoidance of gluten [6]. These observations suggest that gluten sensitivity may be a contributing factor in some cases of childhood NS [4]. We recently described our experience with difficult-to-treat NS in a nonrandom cohort of patients whose clinical behavior improved after starting a gluten-free diet (GFD). Eight children, 7 males and 1 female, age 3–15 years, were placed on a GFD for  $3.4 \pm 4.3$  years (range: 1–14). In each case, there was a clinical improvement, with a decrease in the relapse rate that enabled reduction or discontinuation in steroid dosage [8]. This suggests that in a subset of children with difficult-to-treat NS, elimination of gluten from the diet may reduce the need for potentially toxic immunosuppressive therapies. In patients with celiac dis-

ease (CD), gliadin induces the release of zonulin by the enterocyte, and this molecule in turn disrupts the tight junction between adjacent cells and increases gastrointestinal permeability [9, 10]. The plasma zonulin level is elevated in children with NS and may serve as a biomarker of patients who may be more likely to respond to a GFD [11]. The identification of patients who are more likely to respond to GFD intervention has the potential to align with precision medicine initiatives. Therefore, we conducted an open-label multicenter pilot feasibility study to test the hypotheses that (1) implementation of a GFD is safe and well tolerated and can reduce disease activity in children with difficult-to-treat NS and (2) an elevated plasma zonulin level identifies children with this condition who have a higher likelihood of responding to this dietary intervention.

## Methods

### *Study Design*

This was a multicenter, prospective, open-label, pilot feasibility study to test the efficacy of a GFD in children with difficult-to-treat NS. It was a shared project among the Clinical and Translational Science Awards (CTSA) clinical research units at the 7 participating institutions that collaborated on the retrospective case series of the effect of a GFD in childhood NS [8].

### *Study Population*

Children and adolescents, age 9 months to 18 years, with demonstrated steroid-sensitive NS, namely complete remission of proteinuria in response to standard courses of corticosteroids who then developed difficult-to-treat NS, were eligible for enrollment into the pilot study. This was defined as disease that could not be controlled without incurring intolerable side effects from currently available immunosuppressive agents. Patients with biopsy-proven MCD or focal segmental glomerulosclerosis (FSGS) were eligible as long as they had steroid-sensitive disease. A renal biopsy was not required for enrollment.

Patients were identified using the electronic health record, divisional databases, or community engagement programs. After providing consent, the NS disease activity – number of relapses in the preceding 6 months, FRNS or SDNS status, current medications, and side effects of steroids and other immunosuppressive drugs – were recorded. There was no restriction based on gender or race/ethnicity. The Trial Innovation Network and the Recruitment Innovation Center (Vanderbilt) were engaged for support and assisted with optimizing recruitment planning and strategies, design and production of recruitment materials, and suggestions for community outreach in the geographic region surrounding the participating sites.

### *Study Endpoints*

There were two primary endpoints. The first was the number of patients who achieved a decrease in disease activity, defined as a  $\geq 50\%$  decrease in the relapse rate and/or a reduction by  $\geq 1$  im-

munosuppressive medication assessed after implementation of the GFD for 6 months. The decision to discontinue an immunosuppressive medication was left to the discretion of the site investigator. This response was considered a significant clinical benefit for children with difficult-to-treat NS. Patients who dropped out or who were not compliant with the GFD were viewed as nonresponders in the assessment of the primary outcome. The second primary endpoint was safety, namely the number of adverse events following implementation of the GFD.

#### Study Procedures

Demographic data, including age, sex, race/ethnicity, were collected by review of the electronic medical record. Clinical information, including the NS disease activity, specifically the number of relapses in the preceding 6 months, FRNS or SDNS status, current medications, and side effects of steroids and other immunosuppressive drugs, was recorded. A complete physical examination was done, including height, weight, BMI, assessment of edema, and blood pressure.

A physical examination and clinical assessment, including nutritional evaluation, was done at 1, 3, and 6 months. A follow-up assessment was performed 3 months after discontinuing the GFD.

Immunosuppressive medications prescribed for the treatment of the NS were unchanged for the first month after starting the GFD. They could subsequently be modified at the discretion of the treating physician.

#### Laboratory Procedures

Laboratory testing baseline was performed as part of the routine care of children with difficult-to-treat NS. This included comprehensive metabolic profile, complete blood count, lipid profile, and urine total protein:creatinine ratio in a first morning urine sample. Study-specific blood tests were performed to exclude CD, namely, serum IgA level, tissue transglutaminase IgA, anti-gliadin IgA, and anti-gliadin IgG.

Routine laboratory testing was performed at each follow-up visit. CD testing was done at baseline, month 6, and 3 months after stopping the GFD. In addition, plasma was obtained at baseline and after 6 months of treatment for measurement of zonulin levels. This was done using a commercially available ELISA kit (Immuno-ndiagnostic AG, Stubenwald-Allee 8a, D-64625 Bensheim, Germany) in the NYU Core Laboratory.

#### Study Intervention

After consultation with a registered dietician that included an evaluation of the patient, parents were instructed on how to implement a strict GFD. This included information about foods to avoid, safe foods to have at home, grocery shopping guidance, and meal and snack planning. This procedure was followed because the limited funding for the project precluded provision of the diet to study participants. Patients were treated with the GFD for 6 months. The length of the treatment period reflected a balance between a duration sufficient to document a change in disease activity while avoiding undue burden on study participants. They were seen either in person or via telephone interview by the nutritionist after 2 weeks to verify adherence to the GFD and willingness to remain on the diet for the duration of the trial. A 24-h diet history was collected, and suggestions made for optimal GFD adherence and well-rounded nutritional intake. The assessment did not include any other aspects of the diet including the overall health

**Table 1.** Demographic and clinical characteristics of the study participants

Patient number	Age at baseline	Gender	Ethnicity/race	Diagnosis
01	13	F	N H/L, W	FSGS
02	5	F	N H/L, W	MCD
03	13	F	N H/L, W	MCD
04	5	F	N H/L, W	MCD
05	4	F	N H/L, W	FSGS
06	3	M	N H/L, W	MCD
07	8	F	N H/L, A	MCD
08	16	M	N H/L, W	MCD
09	2	M	N H/L, W	MCD
10	9	F	N H/L, A	FSGS
11	2	M	N H/L, W	MCD
12	2	F	N H/L, W	MCD
13	4	M	N H/L, W	MCD
14	12	M	N H/L, W	MCD
15	13	M	H/L, O	FSGS
16	4	M	H/L, B	MCD
17	2	F	N H/L, W	MCD

FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; NH/L, non-Hispanic/Latino; H/L, Hispanic/Latino; W, White; B, Black; A, Asian.

quality or intake of calories, protein, fat, or sodium. A 5-question survey was administered at 1 month, 3 months, and 6 months following initiation of the GFD to assess adherence. Based on responses, a score 0–5 was assigned with higher scores indicating a greater level of adherence. The adherence was categorized as good (4–5), moderate (3), or questionable ( $\leq 2$ ). Patients who manifested a clinical improvement after 6 months were offered the option of continuing the GFD with follow-up every 3 months.

#### Statistical Methods

In this pilot feasibility study, it was proposed to enroll 30 patients with the expectation that this would represent a sample size sufficient to estimate the effect size of the GFD intervention that could be used in the design of a more definitive randomized clinical trial. Descriptive data are presented as mean  $\pm$  SD. Disease activity during the 6-month treatment period was compared to the 6-month period prior to enrollment. Differences between groups were evaluated with Student's *t* test. Linear regression was used to assess relationships between variables. Results were considered significant if  $p < 0.05$ .

## Results

The pilot feasibility study was open to enrollment from September 7, 2017 until June 16, 2021. Over that period, 17 patients agreed to participate in this open-label pilot feasibility study. Enrollment did not achieve the goal of

**Table 2.** Clinical and laboratory results at baseline and at the end of the 6-month GFD treatment period

ID	Baseline				End of the study period or treatment				
	relapse rate	IS meds, <i>n</i>	UP/C	eGFR	relapse rate	IS meds, <i>n</i>	UP/C	eGFR	remarks
01	SDNS*	2 (P, M)	0.83	110.1	FPRE (not CR)	1 (M dose reduction)	0.44	100	Responder
02	3	2 (P, M)	0.12	149.7	4	1 (P)	0.25	113	Withdrawn
03	3	1 (P)	0.41	166	1	1 (P)	3.43	142	Responder
04	3	2 (P, M)	0.78	105	2	1 (P)	0.23	118	Responder
05	4	2 (P, T)	0.27	84	3	2 (P, T)	2.0	115	Nonresponder
06	4	1 (P)	0.16	98	2	1 (P)	0.2	93	Responder
07	4	2 (P, T)	0.26	116	3	1 (P, T)	10.1	141	Withdrawn
08	2	3 (P, M, R)	0.04	97	2	3 (P, M, T)	0.04	96	Nonresponder**
09	3	1 (P)	0.27	86	3	1 (P)	0.22	95	Nonresponder
10	SDNS*	3 (P, T, M)	0.75	111	0	3 (M, T), D/C P	0.33	89	Responder
11	2	1 (P)	0.28	77	2	2 (P, M)	0.26	87	Withdrawn
12	4	2 (P, M)	19.15	98	3	1 (P, M)	ND	ND	Withdrawn
13	3	1 (P)	0.25	101	2	1 (P)	1.23	106	Nonresponder**
14	0	2 (P, M)	0.18	108	0	2 (P, M dose reduction)	0.13	125	Nonresponder
15	0	3 (P, T, M)	2	ND	0	3 (P, T, M)	ND	ND	Withdrawn
16	2	2 (P, M)	0.11	115	2	2 (P, M)	0.13	119	Nonresponder**
17	2	2 (P, M)	0.23	136	2	3 (P, M)	0.17	75	Nonresponder
Mean ± SD	2.6±1.3	1.9±0.7	1.62±4.6 Median: 0.27	110±23	2.0±1.2	1.8±1.0	1.28±2.52 Median: 0.26	108±19	

P, prednisone; M, mycophenolate mofetil; T, tacrolimus, R, rituximab; ND, not done; FPRE, focal segmental glomerulosclerosis partial remission endpoint, namely, UPC <1.5 g/g and absolute value declined at least 40% from baseline [12]; IS, immunosuppressive. \*These patients had steroid-responsive NS but required maintenance of steroid therapy to control proteinuria. \*\* These patients had a partial response (see Results).

30 patients; however, study was closed at that point because of the adverse impact of the COVID-19 pandemic on clinical trial activity at the participating sites.

Demographic and clinical data of the patient cohort are shown in Table 1. There were 8 boys and 9 girls, age  $7.4 \pm 4.7$  years with a racial distribution of 13 White, 1 Black, and 3 Asian patients. Two patients were Hispanic. Four patients had kidney biopsy-confirmed FSGS. The remaining cases were presumed to be MCD based on the history of sustained steroid responsiveness (Table 1). None of the patients were on a special diet to treat an underlying medical condition.

At the time of enrollment, all but 1 patient were receiving corticosteroids. In addition, 7 children were prescribed mycophenolate mofetil including the child who was not taking prednisone, 3 were on tacrolimus, and 1 adolescent had received rituximab (Table 2).

A total of 12 patients completed the 6-month intervention (Table 2). Five children withdrew prior to the end of the treatment period. In 2 cases, this was due to lack of efficacy of the GFD and in 3 cases at the request of the parents because of perceived inconvenience. The GFD was well tolerated by all participants. There were no clin-

ical or laboratory adverse events attributable to the intervention that prompted early withdrawal from the study. None of the participants had impaired linear growth or weight loss during the 6-month study period.

Overall, the relapse rate was unaltered by the GFD intervention,  $2.6 \pm 1.3$  during the 6-month study period versus  $2.5 \pm 0.7$  in the prior 6 months. However, 5 of 17 (29%) patients satisfied the criterion for a positive response to the GFD based on the defined primary endpoint of either a 50% decline in the relapse rate or the ability to eliminate one immunosuppressive drug from the management regimen. Specifically, 4 patients benefited based on a reduced relapse rate, 1 of whom also had a reduction in immunosuppressive drug dosing (Table 2). One additional patient was able to discontinue one immunosuppressive medication. In 3 cases, there was a decrease in disease severity that did not meet the definition of response to the GFD. One participant responded more quickly to prednisone when it was restarted for relapses, within 1 week versus up to 1 month prior to implementation of the GFD. This was accompanied by an improved sense of well-being. The second child had two relapses of proteinuria during the study, one resolved spontaneously

without the administration of corticosteroids, and a second occurred in the sixth month of the study. In the third case, there were two relapses during the study, one within a month of starting the GFD and a second episode during the fifth month of treatment requiring the administration of corticosteroids. The clinical status of the participants documented at month 6 was sustained for 3 months after discontinuation of the GFD. Of note, 2 patients remained on the GFD after completion of the study.

The GFD was well tolerated by all participants. There were no clinical or laboratory adverse events attributable to the intervention that prompted termination of the GFD or early withdrawal. Results of testing for serum IgA level, tissue transglutaminase IgA, anti-gliadin IgA, and anti-gliadin IgG remained normal in all participants throughout the treatment period.

The total number of patients who completed the nutritional adherence evaluation declined as patients dropped out of the study. All 17 participants completed the survey at month 1, 11 at month 3, and 10 at month 6. Nine patients of the 12 subjects who received the GFD for 6 months completed the questionnaire at all 3 time points. All of these participants had excellent or moderate adherence to the GFD throughout the 6 months except for 1 child with questionable adherence at month 6. It is worth noting that moderate adherence was 47% at month 1, 73% at month 3, and 50% at month 6 among the participants who completed the questionnaire at all 3 time points. Because many of the nonresponders dropped out of the study before completion at 6 months, we evaluated adherence at 3 months instead of after the first month as a reflection of sustained adherence to the nutritional protocol. Among the 11 participants who completed the survey at 3 months, 4 of the 5 responders had moderate adherence and 1 was good. The adolescent with a partial response based on a more rapid response to corticosteroid treatment for relapses was also graded as moderate. Three of the 6 nonresponders had moderate adherence, and 3 had good adherence. There was no difference in adherence to the GFD in responders versus nonresponders or between those who completed the treatment period versus those who dropped out prematurely; however, the number of patients in these subgroups is limited.

The plasma zonulin concentration was measured at both baseline and at study completion in 6 participants. The initial level was  $17.4 \pm 3.2$  pg/mL at the start of the GFD and was elevated ( $>17.5$  pg/mL) in 3 children. Overall, the concentration was unchanged at the end of the treatment period ( $18.9 \pm 5.8$  pg/mL). Among these 6 participants with paired measurements, the baseline plasma

zonulin concentration was  $19.7 \pm 1.7$  versus  $13.4 \pm 0.9$  pg/mL in GFD nonresponders ( $n = 4$ ) versus responders ( $n = 2$ ), respectively,  $p = 0.01$ . There was no difference in the change in the zonulin level over the course of treatment in the responders versus nonresponders to the GFD.

## Discussion

In this multicenter, open-label feasibility study, we documented that 5 out of 17 (29%) of patients with difficult-to-treat NS had a beneficial response to a GFD with either a reduced relapse rate and/or the ability to discontinue  $\geq 1$  immunosuppressive drug. Three additional patients who did not meet the formal definition of benefit reported reduced severity of the NS while on the GFD based on more rapid response to treatment or an increased interval between relapses. The dietary intervention was generally accepted by parents and patients and was not associated with adverse effects. With the support of periodic nutritional counseling, the GFD was feasible in the communities where the patients lived. However, a considerable proportion of subjects did not complete the study (5 out of 17) due to perceived lack of benefit or inconvenience. Adherence to the GFD was generally good, but there was no association between adherence and the clinical response.

The rationale for studying GFD in difficult-to-treat NS is based on prior clinical experience [5–8] and mechanistic studies of podocytes [13, 14]. Podocyte foot processes (FP) are separated by a slit diaphragm and contain an actin cytoskeleton-based contractile apparatus. In MCD and FSGS, early pathological changes in podocytes involve active rearrangement of the FP actin cytoskeleton and the reorganization of the slit diaphragm, leading to simplification of the interdigitating podocyte pattern, a process termed FP effacement [13–15].

Zonulin, which activates protease activated receptor 2 (PAR2), is the molecule that mediates gastrointestinal injury in CD [9, 10]. PAR2 is a member of the G-protein coupled receptor family and is expressed on podocytes. Exposure of podocytes to zonulin induces signaling events in podocytes including ZO-1 phosphorylation and translocation from the cell surface to the nucleus in vitro, changes in the actin cytoskeleton, and disruption of cell-cell junctions [15, 16]. Addition of zonulin to culture media impairs podocyte cell migration and increases paracellular flux [unpubl. observation]. PAR2 knockdown protects against these alterations. A zonulin knock-in mouse model with elevated serum zonulin levels develops

albuminuria [unpubl. observation]. Implementation of a GFD may prevent the adverse effects of zonulin in the podocyte. Consistent with this hypothesis and our observations in children with difficult-to-treat NS, initiation of a GFD in patients with newly diagnosed CD is associated with a reduction in microalbuminuria [17].

Elevated plasma zonulin levels, which is supportive of a diagnosis of CD, may be a laboratory feature that characterizes patients with difficult-to-treat NS. In a cross-sectional study of pediatric patients enrolled in the NEPTUNE observational cohort study, the plasma concentration of zonulin was higher in children with NS compared to healthy age-matched controls [11]. The levels were unrelated to age, gender, the presence of MCD versus FSGS, steroid responsiveness, or the use of second-line immunosuppressive medications. In addition, there was no relationship between plasma zonulin levels and the absolute or percentage change in proteinuria from enrollment into the NEPTUNE observational study until the time of the zonulin assay. In contrast to our hypothesis, our results and the NEPTUNE data suggest that an elevated plasma zonulin levels may predict nonresponse to a GFD. Additional work is needed to assess the role of zonulin in NS and the application of plasma zonulin concentration as a biomarker to identify children who are more likely to benefit from the dietary intervention.

It is important to recognize that gliadin-induced release of zonulin by enterocytes is likely to be associated with higher local levels in the gastrointestinal lumen than in Bowman's space. This reflects the passage of the molecule through the portal circulation and filtration across the glomerular capillary wall before reaching its putative renal site of action, namely, the podocyte. Consequently, there may be less disruption of the tight junctions between adjacent visceral glomerular epithelial cells compared to diet-induced alteration in the integrity of enterocytes in the gastrointestinal epithelium [18]. This may explain why the GFD was not uniformly effective and, when it was, why it appeared to modulate disease severity rather than achieve a sustained complete remission. Thus, although most of the responders continued to have relapses, the GFD reduced the frequency and enhanced responsiveness to corticosteroids and second-line immunosuppressive agents. The reason for the difference between this response and the complete remission of NS in previous dietary intervention studies [5–7] is unclear. Ongoing efforts by other investigators to assess the effect of a GFD as a treatment for glomerular diseases will help determine the place of this dietary intervention for the treatment of childhood NS [19, 20].

Putting this pilot study in perspective, the COVID-19 pandemic has highlighted the importance of compromised immunity as risk factors for severe viral infection. Although the use of immunosuppressive agents including rituximab was not associated with increased frequency or severity of COVID-19 infection in pediatric patients with NS [21], it is reasonable to attempt to control the kidney disease with interventions that do not interfere with the immune response. A 3-month trial of a GFD may be a useful adjunct to the treatment of difficult-to-treat NS before implementing a second-line immunosuppressive agent.

There have been reports that use of a GFD can be associated with a variety of nutritional deficiencies [22]. Our patients received regular counseling to ensure that they were receiving a balanced healthy diet. Moreover, we did not document impaired growth. However, the duration of treatment was modest and surveillance studies were not performed to detect specific nutritional disturbances. In future studies of a GFD in childhood NS, systematic assessment of nutritional status should be incorporated into the protocol.

We acknowledge some limitations of this study. In the absence of routine screening of all potential participants at each site, the sample may be biased by inclusion of patients deemed by the investigator to be more likely to implement a GFD. The treatment was open label, and discontinuation of immunosuppressive medications was implemented at the discretion of the site investigator. Thus, confirmation of efficacy of a GFD in a randomized clinical trial is needed. Complete adherence to the GFD, which is required in the treatment of CD, was not observed in the trial participants. The moderate degree of adherence to the GFD provides a realistic estimate of acceptance of the intervention in a circumstance where the efficacy is not clearly established as it is in patients with CD. Consequently, the full effect of a GFD cannot be ascertained from this study. The number of patients who discontinued the GFD and withdrew before the end of the 6-month treatment period is another drawback of the study. In this regard, most of the patients were young, and it is unclear if a GFD is feasible in adolescents and older patients with NS. The small sample size limited our ability to identify specific clinical factors associated with efficacy of the GFD.

In conclusion, the results of this pilot feasibility study suggest that implementation of a common dietary modification, a GFD, can be accomplished safely and may attenuate disease activity in approximately one-third of patients with difficult-to-treat NS. A 3-month trial of a GFD

may be a useful approach to the management of affected children before implementing a second-line immunosuppressive agent. Additional work is needed to determine whether the plasma zonulin concentration is a biomarker that can be used to determine the likelihood of response to the dietary intervention. The growing medical use of and greater access to gluten-free food items underscore the feasibility of this dietary approach. We recommend the conduct of a multicenter randomized clinical trial to confirm the findings in this open-label pilot study, to further define the predictive power of zonulin as a biomarker and to determine the efficacy of a GFD in the management of children with difficult-to-treat NS.

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### Statement of Ethics

The study was reviewed and approved by a central IRB, CIRB #171516, Reliance Exchange at Vanderbilt University Medical Center. It was also reviewed and approved by the NYU Grossman School of Medicine IRB, NYU IRB #17-01307, and the local IRB of each participating site. In this pilot study using pediatric participants, written informed consent was obtained from their parent/legal guardian/next of kin to participate in the study. Assent was also obtained from the participant in accord with age-appropriate guidelines at each participating site. The clinical research complied with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The clinical trial was conducted as part of the Trial Innovation Network that is supported by NCATS and housed at Duke.

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### Conflict of Interest Statement

None of the authors have a conflict of interest relevant to this study.

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### Author Contributions

Tarak Srivastava, Katherine M. Dell, and Frederick J. Kaskel enrolled participants, participated in data analysis, and preparation of the manuscript; Kevin V. Lemley, Debbie S. Gipson, and Christian Faul participated in data analysis and preparation of the manuscript; Kevin Meyers participated in data analysis and preparation of the manuscript; Ayelet Goldhaber performed the nutritional assessments, evaluated adherence to the GFD, and participated in data analysis and preparation of the manuscript; Laura-Jane Pehrson assisted in preparation of study materials and regulatory approvals, data analysis, and preparation of the manuscript; Howard Trachtman conceived the study, supervised patient enrollment and management, was responsible for regulatory approval, and led the data analysis and preparation of the manuscript.

### Data Availability Statement

The data from this study have not yet been uploaded into a publicly accessible data repository. They are available on request to the corresponding author.

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