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Published in:
Kidney International

DOI:
10.1016/j.kint.2023.02.022

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
CNI in Monogenic SRNS Study Investigators, Malakasioti, G., Iancu, D., Milovanova, A., Tsygin, A., Horinouchi, T., Nagano, C., Nozu, K., Kamei, K., Fujinaga, S., Iijima, K., Sinha, R., Basu, B., Morello, W., Montini, G., Waters, A., Boyer, O., Yıldırım, Z. Y., Yel, S., ... Tullus, K. (2023). A multicenter retrospective study of calcineurin inhibitors in nephrotic syndrome secondary to podocyte gene variants. *Kidney International*, *103*(5), 962-972. Advance online publication. https://doi.org/10.1016/j.kint.2023.02.022

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A multicenter retrospective study of calcineurin inhibitors in nephrotic syndrome secondary to podocyte gene variants

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While 44-83% of children with steroid-resistant nephrotic syndrome (SRNS) without a proven genetic cause respond to treatment with a calcineurin inhibitor (CNI), current guidelines recommend against the use of immunosuppression in monogenic SRNS. This is despite existing evidence suggesting that remission with CNI treatment is possible and can improve prognosis in some cases of monogenic SRNS. Herein, our retrospective study assessed response frequency, predictors of response and kidney function outcomes among children with monogenic SRNS treated with a CNI for at least three months. Data from 203 cases (age 0-18 years) were collected from 37 pediatric nephrology centers. Variant pathogenicity was reviewed by a geneticist, and 122 patients with a pathogenic and 19 with a possible pathogenic genotype were included in the analysis. After six months of treatment and at last visit, 27.6% and 22.5% of all patients respectively, demonstrated partial or full response. Achievement of at least partial response at six months of treatment conferred a significant reduction in kidney failure risk at last follow-up compared to no response (hazard ratio [95% confidence interval] 0.25, [0.10-0.62]). Moreover, risk of kidney failure was significantly lower when only those with a follow-up longer than two years

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Received 11 May 2022; revised 4 February 2023; accepted 16 February 2023; published online 8 March 2023

were considered (hazard ratio 0.35, [0.14-0.91]). Higher serum albumin level at CNI initiation was the only factor related to increased likelihood of significant remission at six months (odds ratio [95% confidence interval] 1.16, [1.08-1.24]). Thus, our findings justify a treatment trial with a CNI also in children with monogenic SRNS.

Kidney International (2023) **103,** 962–972; https://doi.org/10.1016/j.kint.2023.02.022

KEYWORDS: calcineurin inhibitors; kidney failure; monogenic steroid-resistant nephrotic syndrome

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Lay Summary

Calcineurin inhibitors (CNI) are immunosuppressive medications very efficacious in childhood steroid-resistant nephrotic syndrome (SRNS). However, there is a subgroup of children with genetic mutations responsible for the disease in whom CNI are considered non-efficacious and are contraindicated. Yet, to date, there are no studies that have specifically addressed the efficacy of CNI in genetic SRNS and how they could affect long-term kidney prognosis. We retrospectively assessed the records of 141 children with genetically confirmed SRNS from 37 international pediatric nephrology centers who had received CNI treatment. Approximately 1 in 4 children showed response to therapy, but more importantly, children responding to this treatment had a 75% lower risk for kidney failure compared with those who did not respond. Our study is the first to show that CNI can actually work in children with genetic SRNS and increase kidney survival, reducing the need for kidney replacement therapy.

Steroid-resistant nephrotic syndrome (SRNS) accounts for 10%–15% of pediatric cases with nephrotic syndrome (NS). With the widespread use of sequencing technologies, genetic defects have been reported in up to one-third of cases with SRNS.[1] Identifying an associated genetic diagnosis has major therapeutic implications because the 2020 International Pediatric Nephrology Association Clinical Practice Recommendations for SRNS recommend calcineurin inhibitors (CNI) as first-line immunosuppressive agents, as they are efficacious in 44%–83% of cases, but state that this treatment should not be continued in patients with monogenic SRNS.[2–5]

However, there is emerging evidence that CNI resistance in monogenic SRNS is not a dogma. In a study by Buscher *et al.*,[4] a favorable response rate of 19% was identified among 131 monogenic SRNS cases, and this finding was in agreement with the results of immunosuppressive treatment for those patients identified in the PodoNet registry.[6] In our recent systematic review, we identified 22 published studies including 178 patients with monogenic SRNS who were treated with CNI.[7] Of those, 35% responded to CNI therapy, and more importantly, such a response conferred a 40% reduction in the risk of progression to kidney failure compared with cases without remission.[7] However, this study had important limitations such as the inclusion of patients from case series and case reports likely subject to positive publication bias and the lack of clarity in defining partial response. Given the potential side effects of immunosuppression, a larger study is necessary to explore the role of treatment with a CNI in monogenic SRNS in a more objective and systematic way.

Hence, the aims of the present multicenter, retrospective study were to: (i) determine the CNI response rate in the largest to date monogenic pediatric SRNS cohort; (ii) assess the effect of CNI treatment on long-term kidney function preservation; and (iii) explore clinical, laboratory, histopathologic, and genetic predictors of response.

METHODS

Patient eligibility and data collection

An email was sent to the membership of the European Society for Paediatric Nephrology and the International Pediatric Nephrology Association, inviting clinicians to provide data on patients who fulfilled one of the following eligibility criteria: (i) children with SRNS aged 0–18 years at the onset of NS carrying podocyte gene variants and treated with a CNI (either tacrolimus or ciclosporin A) for at least 3 months; or (ii) children with monogenic SRNS (i.e., congenital or syndromic NS) who were never treated with corticosteroids but who were administered a CNI. Between September 2020 and March 2021, we retrospectively collected anonymous data of eligible patients followed up in pediatric nephrology centers from Europe, North and South America, Asia, and Oceania.

Anonymized demographic, clinical, biochemical, histopathologic, genetic, and treatment data from each subject were reviewed at different time points, specifically: (i) at diagnosis; (ii) at CNI treatment initiation; (iii) at 6, 12, and 24 months into CNI treatment; and (iv) at last visit available or at initiation of kidney replacement therapy (whichever occurred first).

This study was performed according to the Declaration of Helsinki and was approved by the local ethics committees as per each center's legal requirements. Informed consent was obtained from patients or carers by researchers at each institution in accordance with local ethics committee regulations.

Definitions

Patients' clinical presentation was classified in 1 of 3 patterns: (i) congenital NS (CNS) if disease onset was within first 3 months of life; (ii) infantile NS if onset between 3 and 12 months of life; and (iii) overt NS defined as spot urine protein-to-creatinine (UPC) ratio >2 mg/mg (>200 mg/mmol) or 24-hour urine protein >40 mg/m^2/h (i.e., >1 g/m^2/d), hypoalbuminemia (serum albumin <25 g/l), and peripheral edema beyond infancy.

Proteinuria was expressed as the UPC ratio from random urine samples. When only 24-hour urine protein excretion was available, conversion to UPC was carried out as previously described by Abitbol *et al.*[8] According to Schneider *et al.*,[9] the UPC ratio was calculated from spot urine albumin-to-creatinine ratios. The modified Schwartz formula was used for estimated glomerular filtration rate calculation.[10] In terms of response to treatment at the specified

time points, patients were classified according to International Pediatric Nephrology Association clinical practice recommendations as follows: (i) *full responders* if the UPC ratio at the respective time point was ≤0.2 mg/mg (≤20 mg/mmol), (ii) *partial responders* if the UPC ratio at each time point was >0.2 mg/mg (>20 mg/mmol) but <2 mg/mg (<200 mg/mmol) and serum albumin level, when available, was ≥30 g/l; or (iii) *nonresponders* if the UPC ratio or serum albumin achieved at each time point did not meet any of the above criteria.

For those patients who progressed to kidney failure, the end of the follow-up period was defined as the time point that patients were started on kidney replacement therapy. In all other cases, follow-up duration was as per patient records until the last clinic visit.

The reported podocyte gene variants were assessed by an accredited geneticist (DI) according to the American College of Medical Genetics and Genomics and the Association for Clinical Genomic Science criteria.[11,12] Cases were further categorized based on the anticipated contribution of their genotype to the SRNS phenotype as previously described.[7] In brief, for autosomal dominant traits, the genotype's contribution to the phenotype was classified as follows: (i) *benign* in patients with "benign/likely benign" variants, (ii) *uncertain* in cases with variants of "unknown significance" (VUS), (iii) *possibly pathogenic* if "likely pathogenic" variant, or (iv) *pathogenic* in carriers of "pathogenic" variants. We opted for this classification to highlight the difference between class 4 (likely pathogenic) and class 5 (pathogenic) variants. In clinical settings, both class 4 and class 5 variants are considered as actionable and relevant for clinical management and genetic counseling in families. For autosomal recessive traits, the allele combination effect on the phenotype was classified as follows: (i) *benign* for patients with "benign/likely benign" variants in allele 1 and "benign/likely benign/VUS" in allele 2, (ii) *unknown* in case of 2 "VUS" in different alleles, (iii) *possibly pathogenic* if "likely pathogenic/pathogenic" variant in allele 1 and "VUS" in allele 2, or (iv) *pathogenic* if "likely pathogenic/pathogenic" variant in allele 1 and "likely pathogenic/pathogenic" variant in allele 2. According to the Association for Clinical Genomic Science recommendations, we considered the association between a likely pathogenic variant on one allele and a "hot VUS" on the other allele as a *possibly pathogenic* allele combination effect.[12] The decision was supported using the available clinical, biochemical, and bioinformatic evidence that suggested pathogenicity but without reaching the level required for a classification as likely pathogenic. The term "hot VUS," as indicated in the Association for Clinical Genomic Science 2020 recommendations, was applied to identify those variants that are likely to be upgraded to "likely pathogenic" if additional evidence becomes available.[12] The type of each variant according to their translational effect (nonsense, missense, splice-site, or frameshift) was also assessed.

Cases falling under the category "*benign* genotype" or "genotype with *unknown* effect on phenotype" were excluded from the primary analysis. Further subgroup analyses were undertaken for patients with *pathogenic* genotype after exclusion of subjects with *possibly pathogenic* genotype.

Statistical analysis

Continuous variables were reported as median (range). Categorical data were expressed as percentages. Groups were compared in terms of categorical characteristics using the χ^2 test and in terms of continuous variables using the Kruskal-Wallis or the Mann-Whitney test, depending on the number of groups. To detect the predictors of at least partial response at 6 months, we initially developed a multivariable logistic regression model of variables of clinical interest and then used a stepwise backward elimination process to identify significant associations. The variables considered were as follows: mode of presentation, histopathology on kidney biopsy, serum albumin at CNI initiation, podocyte protein gene group function (genes encoding for slit diaphragm vs. nuclear, actin cytoskeleton, mitochondrial, glomerular basement membrane, or other proteins), renin-angiotensin-aldosterone system (RAAS) blockade, CNI type, and delay in CNI initiation from diagnosis. Removal testing was based on the probability of the likelihood-ratio statistic according to the maximum partial likelihood estimates. Because of the diversity of

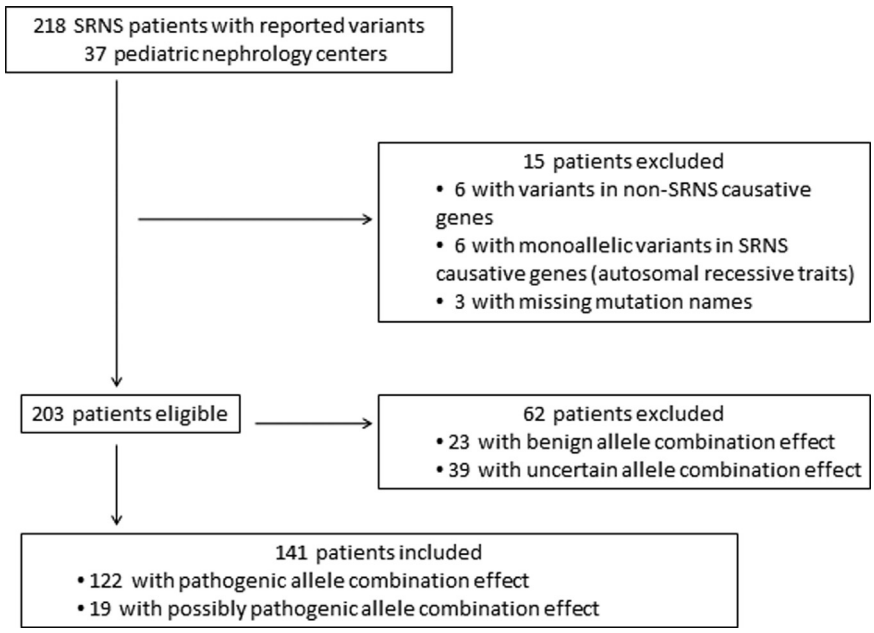

Figure 1 | Patient selection flowchart. SRNS, steroid-resistant nephrotic syndrome.

identified gene variants, direct comparisons were only performed for the 3 predominant genes (*NPHS1*, *NPHS2*, and *WT1*). Cumulative kidney survival across different levels of treatment response at 6 months was analyzed by Kaplan-Meier survival curves and log-rank tests. Cox regression analysis was also performed to predict risk (expressed as hazard ratio [HR]) for kidney failure progression according to response to CNI therapy at 6 months. Two-tailed *P* values lower than 0.05 were defined as statistically significant.

RESULTS

Patient characteristics

Data of 218 patients were collected from 37 pediatric nephrology centers. After the exclusion of cases with missing variant details, variants in genes not associated with SRNS or monoallelic variants (for autosomal recessive traits), 203 children underwent variant pathogenicity assessment and cases with the *benign* and *unknown* allele combination effect were excluded from further analysis; 141 cases with either *pathogenic* (N = 122) or *possibly pathogenic* (N = 19) genotype were finally included in the study. The patient selection flowchart is shown in Figure 1. Seventeen patients have been described in previous publications (Supplementary Table S1).

Demographic, clinical, genetic, laboratory, and biopsy data at diagnosis are summarized in Table 1. Overt NS was the predominant presentation pattern, with focal segmental glomerulosclerosis being the most common biopsy finding. The median follow-up duration from clinical presentation was 55 months.

Variants in SRNS genes

Variants in 22 SRNS causative genes were identified (Table 1). The majority of variants were detected in *NPHS2*, *WT1*, and *NPHS1*, with missense changes being the most frequent type of variants, followed by nonsense and frameshift for *NPHS1* and *NPHS2* and splice-site variants for *WT1*. A variant list is provided in Supplementary Table S2. Patient country of origin, genotype, variant class, and type are presented in Supplementary Table S1. Variants classified as "hot VUS" inherited in trans with a convincing variant are listed in Supplementary Table S3.

Genetic diagnosis followed clinical diagnosis by a median of almost 3 years. Patient numbers according to the era of clinical presentation were as follows: (i) before 2010 (N = 40 of 138), (ii) between 2010 and 2015 (N = 49 of 138), and (iii) after 2015 (N = 49 of 138). The lag between clinical and genetic diagnosis was significantly shorter over time, with a median time difference of 92 (2.7–245.1), 40 (0.8–110.4), and 8.6 (1.1–54.2) months for children diagnosed before 2010, between 2010 and 2015, and after 2015, respectively ($P < 0.001$).

Response to treatment

Treatment data are summarized in Table 2. In 67% and 82% of patients, corticosteroids and RAAS blockers were coadministered with CNI, respectively.

Ciclosporin and tacrolimus starting doses were 3–5 mg/kg/day and 0.1–0.2 mg/kg/day, respectively. Ciclosporin was the predominant CNI, and children were treated for a median of 1.5 years, with primary CNI resistance being the commonest reason for their discontinuation. The median time elapsed

Table 1 | Patient demographic, clinical, genetic, biochemical, and histologic characteristics

Characteristic	Possibly pathogenic and pathogenic genotype (N = 141)	Pathogenic genotype (N = 122)
Age at presentation, mo	34 (0–193)	31.5 (0–193)
Follow-up (from clinical presentation), mo	55 (5.5–243.6)	54.5 (7.6–243.6)
Female	81 (57.4)	72 (59)
Ethnicity		
Caucasian	78 (55.3)	71 (58.2)
African American	1 (0.7)	1 (0.8)
Asian	57 (40.4)	46 (37.7)
Hispanic	2 (1.4)	2 (1.6)
Other	3 (2.1)	2 (1.6)
Presentation		
CNS	15 (10.6)	15 (12.3)
INS	16 (11.3)	15 (12.3)
Overt NS	110 (78)	92 (75.4)
Family history of NS/CKD	30 (21.3)	28 (23)
Time between clinical and genetic diagnosis, mo	33.2 (0.8–245)	28.4 (0.8–229.3)
Gene mutated		
NPHS1	15 (10.6)	14 (11.5)
NPHS2	49 (34.8)	48 (39.3)
WT1	15 (10.6)	12 (9.8)
INF2	8 (5.7)	6 (4.9)
SMARCAL1	6 (4.3)	5 (4.1)
ADCK4 (*COQ8B*)	7 (5)	4 (3.3)
COQ6	6 (4.3)	5 (4.1)
LMX1B	5 (3.5)	4 (3.3)
NUP93	4 (2.8)	4 (3.3)
ACTN4	4 (2.8)	0
COL4A5	4 (2.8)	4 (3.3)
NUP107	3 (2.1)	3 (2.5)
PLCE1	3 (2.1)	1 (0.8)
COL4A3	2 (1.4)	2 (1.6)
DGKE	2 (1.4)	2 (1.6)
OCRL	2 (1.4)	2 (1.6)
CD2AP	1 (0.7)	1 (0.8)
CD46	1 (0.7)	1 (0.8)
CRB2	1 (0.7)	1 (0.8)
MYO1E	1 (0.7)	1 (0.8)
PAX2	1 (0.7)	1 (0.8)
TPRKB	1 (0.7)	1 (0.8)
Biopsy		
MCD	28 (19.9)	27 (22.1)
FSGS	88 (62.4)	75 (61.5)
DMS	11 (7.8)	7 (5.7)
Other	11 (7.8)	10 (8.2)
Not done	3 (2.1)	3 (2.5)
Laboratory findings at diagnosis		
sAlbumin, g/l	23 (7–45)	22 (8.4–45)
UPCR, mg/mg	5.2 (0.8–81)	5.2 (0.8–81)
eGFR, ml/min per 1.73 m^2	116.7 (37–564)	118.5 (37–402)
Kidney outcome		
Normal kidney function	41 (29.3)	38 (31.4)
CKD stage 2–4	39 (27.9)	34 (28.1)
Kidney failure	60 (42.9)	49 (40.5)

CKD, chronic kidney disease; CNS, congenital nephrotic syndrome; DMS, diffuse mesangial sclerosis; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; INS, infantile nephrotic syndrome; MCD, minimal change disease; NS, nephrotic syndrome; sAlbumin, serum albumin; UPCR, urine protein-to-creatinine ratio. Data are presented as n/total (%) or median (range).

Table 2 | Treatment characteristics

Characteristic	Possibly pathogenic and pathogenic genotype (N = 141)	Pathogenic genotype (N = 122)
Prior IS		
None	15 (9.9)	9 (7.4)
Prednisolone	59 (41.8)	51 (41.8)
Prednisolone + MP pulses	58 (41.1)	52 (42.6)
CS + CPA	10 (7.1)	10 (8.2)
Concomitant CS use	94 (66.7)	78 (63.9)
ACEi/ARB use	116 (82.3)	98 (80.3)
CNI		
Ciclosporin	101 (71.6)	86 (70.5)
Tacrolimus	32 (22.7)	29 (23.8)
Both	8 (5.7)	7 (5.7)
Follow-up, from CNI initiation, mo	42.1 (4.1–175.8)	41.6 (5.7–166.1)
Time of CNI initiation after diagnosis, mo	4.2 (0–185.7)	4.6 (0–185.7)
CNI treatment duration, mo	18 (2.8–147.6)	18 (2.8–147.6)
Time from CNI discontinuation at last visit, mo	19 (0–137)	25.7 (0–137)
Indication for CNI		
Primary CS resistance	136 (97.1)	119 (97.5)
Secondary CS resistance	4 (2.9)	3 (2.5)
Laboratory findings at CNI initiation		
sAlbumin, g/l	24 (7–48.2)	24 (9–48.2)
UPCR, mg/mg	5.5 (0.5–194.4)	5.4 (0.5–194.4)
eGFR, ml/min per 1.73 m^2	118.9 (18–531)	118.1 (18–531)
Reason for CNI discontinuation		
No response	78/107 (72.9)	69/90 (76.7)
Secondary resistance	2/107 (1.9)	1/90 (1.1)
Confirmation of monogenic SRNS	2/107 (1.9)	2/90 (2.2)
Nephrotoxicity	12/107 (11.2)	8/90 (8.9)
Long-term remission	5/107 (4.7)	5/90 (5.6)
Kidney failure	5/107 (4.7)	3/90 (3.3)
Other	3/107 (2.8)	2/90 (2.2)
CNI-responsive recurrences within patients with at least partial response at 6 mo	15/27 (55.6)	13/23 (56.5)
Recurrences after CNI discontinuation (only patients with long-term remission)	0/5	0/5
Patients maintained on CNI		
At 6 mo	124/141 (87.9)	108/122 (88.5)
At 12 mo	87/141 (61.7)	74/122 (60.7)
At 24 mo	57/141 (40.4)	46/122 (37.7)
At last visit	34/141 (24.1)	32/122 (26.2)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CNI, calcineurin inhibitor; CPA, cyclophosphamide; CS, corticosteroids; eGFR, estimated glomerular filtration rate; IS, immunosuppression; MP, methylprednisolone; sAlbumin, serum albumin; SRNS, steroid-resistant nephrotic syndrome; UPCR, urine protein-to-creatinine ratio.
Data are presented as n/total (%) or median (range).

between initial presentation and CNI initiation was 4.2 months and was found to reduce with advancing era (median time difference between clinical diagnosis and CNI onset of 16.4, 4, and 2.2 months for those diagnosed before 2010, between 2010 and 2015, and after 2015, respectively; $P < 0.001$). Individual patient timeline including age at presentation, time until genetic diagnosis and CNI initiation, response to treatment at the various time points, duration of follow-up, and estimated glomerular filtration rate at last visit are presented in Supplementary Table S4.

The rates of response to CNI at 6, 12, and 24 months from treatment initiation and at last visit, as well as drug and serum albumin levels, are presented in Table 3. In summary, at 6 months from CNI introduction, 27.6% of children (data available for 127 of 141 subjects) demonstrated at least partial response (6.3% full response and 21.3% partial response). However, the drug had been discontinued in only 12.6% of children at that time despite many of them (72.4% of the cohort) having not achieved remission.

A total of 29% (29 of 101) and 38% (6 of 16) of children with overt NS and infantile NS, respectively, demonstrated at least partial response after 6 months of treatment, whereas none of the patients with CNS had responded. Response data at 6 months as well as baseline characteristics and kidney outcome for children with variants in the 3 predominant genes (*NPHS1*, *NPHS2*, and *WT1*) are presented in Table 5.

In terms of stability of previously attained response, 56% of patients who demonstrated at least partial response at 12 months maintained it at 24 months and last visit, respectively. A total of 50% (N = 16 of 32) and 48.1% (N = 13 of 27) of fully or partially responding children at 12 and 24 months, respectively, had experienced no more than 1 recurrence that remitted with

Table 3 | Response to CNI therapy at various time points

	Possibly pathogenic and pathogenic genotype				Pathogenic genotype			
Time point from treatment onset	Full response	Partial response	No response	*P*	Full response	Partial response	No response	*P*
6 mo	**N = 8/127**	**N = 27/127**	**N = 92/127**		**N = 7/110**	**N = 22/110**	**N = 81/110**	
CNI level								
Ciclosporin C0 (ng/ml)	103 (68–152)	97 (51–315)	88 (22–596)	ns	107 (68–152)	99 (51–315)	91 (22–596)	ns
Ciclosporin C2 (ng/ml)	403	312 (307–528)	698 (454–735)	ns	403	528	733 (454–735)	ns
Tacrolimus C0 (ng/ml)	NA	8 (7.8–9.1)	6.2 (4.1–11.9)	0.004	NA	8 (7.8–9.1)	6 (4.1–8.1)	0.001
sAlbumin (g/l)	37 (30–45)	38 (31–45.4)	24 (4.3–41.2)	<0.001	37 (30–45)	38 (33–45.4)	23 (4.3–41.2)	<0.001
12 mo	**N = 8/76**	**N = 24/76**	**N = 44/76**		**N = 7/64**	**N = 20/64**	**N = 37/64**	
CNI level								
Ciclosporin C0, ng/ml	57 (52–125)	97 (76–163)[a]	74 (10–123)[a]	0.012	61 (52–125)	109 (78–163)[b]	71 (10–120)[b]	0.006
Ciclosporin C2, ng/ml	550	497 (272–1036)	507 (190–840)	ns	550	565 (428–1036)	540 (190–840)	ns
Tacrolimus C0, ng/ml	6.7	8.4 (6.7–9.3)	6.7 (2.5–11.5)	ns	6.7	8.4 (6.7–9.3)	6.7 (2.5–11.5)	ns
sAlbumin (g/l)	36 (29–43)	36.8 (32–44)	24.5 (7–42.3)	<0.001	36 (29–43)	36.8 (33–44)	26 (7–42.3)	<0.001
24 mo	**N = 6/48**	**N = 21/48**	**N = 21/48**		**N = 5/40**	**N = 18/40**	**N = 17/40**	
CNI level								
Ciclosporin C0, ng/ml	63.5 (36–100)	78 (28–158)	67 (21–139)	ns	59 (36–100)	64 (28–158)	49 (21–139)	ns
Ciclosporin C2, ng/ml	675	360	548 (445–743)	ns	675	360	600 (456–743)	ns
Tacrolimus C0, ng/ml	NA	7.2 (3.4–9.3)	5.4 (1.9–7.2)	ns	NA	7.8 (3.4–9.3)	6.1 (1.9–7.2)	ns
sAlbumin (g/l)	35.2 (25–44)	36.1 (31–43)	26 (12.8–40)	<0.001	38 (31–44)	36.5 (31–43)	28 (12.8–40)	<0.001
Last visit	**N = 5/129**	**N = 24/129**	**N = 100/129**		**N = 4/111**	**N = 23/111**	**N = 84/111**	
CNI level								
Ciclosporin C0, ng/ml	52 (33–72)	106 (62–120)[c]	36 (10–85)[c]	0.042	52 (33–72)	106 (62–120)	41 (10–85)	ns
Ciclosporin C2, ng/ml	NA	426	469 (110–827)	ns	NA	NA	469 (110–827)	ns
Tacrolimus C0, ng/ml	NA	8.0 (3.8–11.2)	5.2	ns	NA	8.0 (3.8–11.2)	5.2	ns
sAlbumin (g/l)	39 (33–42.8)	37 (30–41.7)	25 (8.8–44)	<0.001	39 (33–42.8)	37.3 (30–41.7)	25 (8.8–44)	<0.001

CNI, calcineurin inhibitor; NA, not applicable; ns, not significant; sAlbumin; serum albumin.
[a]$P = 0.004$ for the comparison between patients with nonresponse and partial response at 12 months.
[b]$P = 0.001$ for the comparison between patients of pathogenic genotype with nonresponse and partial response at 12 months.
[c]$P = 0.018$ for the comparison between patients with nonresponse and partial response at last visit.
Data are presented as n/total (%) or median (range).
At 6 months and last visit, analysis includes all patients with available data to assess response. At 12 and 24 months, analysis includes only patients still receiving a CNI at this time point with available data to assess response. At last visit, kidney failure patients (n = 60) are included in the response assessment and considered as nonresponders. Thirty-nine non–kidney failure patients were also nonresponsive to treatment at this time point.

treatment escalation. The remaining subjects relapsed on average 3 times (range: 2–6) during the observation period.

At last visit, data sufficient to assess response to treatment were available for 129 of 141 children. Twenty-two percent (25 of 129) of children maintained at least partial remission (full and partial remission in 3.9% and 18.6%, respectively). The median observation time between CNI initiation and last visit was 42.1 months (interquartile range: 20.1–65.6 months) and was comparable between patients with no response and at least partial response (42.3 vs. 41.6 months; $P > 0.05$). At last visit, 62.8% (81 of 129) of children had chronic kidney disease stage 3 or higher; of these, 88.9% (72 of 81) exhibited no response to treatment compared with 58.3% (28 of 48) of those with chronic kidney disease stage 1–2 ($P < 0.001$). Of these, 108 (84%) received RAAS inhibition, with 23 (21.3%) demonstrating at least partial response. Similarly, 28.6% (6 of 21) not receiving RAAS inhibition attained at least partial response ($P = 0.47$).

For the subgroup of children with *pathogenic* genotype (N = 122), 26.4% (29 of 110 patients with available data) and 24.3% (27 of 111 patients with available data) of participants demonstrated at least partial response after 6 months of treatment and at last visit, respectively (full response 6.4% [7 of 110] and 3.6% [4 of 111]; partial response 20% [22 of 110] and 20.7% [23 of 111]).

CNI treatment effect and kidney function outcome

After a median total follow-up time of 55 months, 43% of children developed kidney failure, whereas 29.3% had normal kidney function (Table 1). Cox regression survival models were used to assess the impact of successful CNI therapy on the long-term kidney outcome. The achievement of at least partial response at 6 months of treatment for all patients and for those who had at least 2 years of follow-up from treatment initiation (N = 91) carried a 75% and 65% lower risk of kidney failure, respectively (HR: 0.25, 95% confidence interval [CI]: 0.10–0.62; $P = 0.003$ and HR: 0.35, 95% CI: 0.14–0.91; $P = 0.03$).

Figure 2 depicts the corresponding Kaplan-Meier kidney survival curves stratified by the level of response to CNI at 6 months for any follow-up duration after CNI initiation (Figure 2a) and for those with a follow-up of at least 2 years after CNI initiation (Figure 2b). Time to kidney failure was significantly longer for children with at least partial response ($P = 0.001$ and $P = 0.02$, respectively).

Results did not change when only patients with *pathogenic* genotype were considered: HR: 0.2, 95% CI: 0.06–0.63 ($P = 0.006$) for those with any follow-up duration (N = 110) and HR: 0.27, 95% CI: 0.08–0.89 ($P = 0.03$) for those with at least 24 months of follow-up (N = 80).

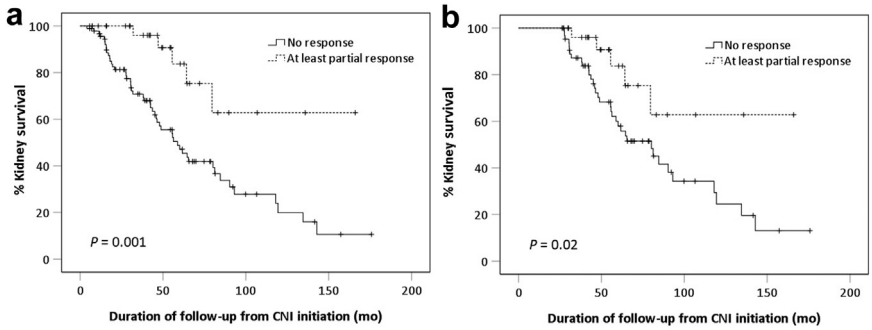

Figure 2| (a) Kaplan-Meier plot of time to kidney failure according to response at 6 months for all subjects. (**b**) Kaplan-Meier plot of time to kidney failure according to response at 6 months for patients with a follow-up of at least 24 months from treatment initiation. CNI, calcineurin inhibitor.

Factors predicting efficacious CNI therapy at 6 months

Data sufficient to assess response at 6 months were available for 127 of 141 children. Of the various clinical parameters tested in a backward stepwise logistic regression model, only serum albumin level at CNI initiation was associated with at least partial response at 6 months (odds ratio: 1.16, 95% CI: 1.09–1.24; $P < 0.001$; Table 4). Results from the respective analysis in the subgroup of children with a *pathogenic* genotype were similar and are provided in Supplementary Table S5.

DISCUSSION

In this study, we demonstrated that 6.3% and 21.3% of children with proven monogenic SRNS had a full or partial response, respectively, at 6 months of treatment with a CNI. More importantly, this favorable response to treatment at 6 months was associated with a 75% and 65% lower risk of progressing to kidney failure if any follow-up duration and if only follow-up of at least 2 years were considered, respectively.

We have thus been able to replicate findings of our previous systematic literature review in terms of frequency of response to a CNI in monogenic SRNS.[7] This remission was sustained as only 2 patients developed secondary resistance and 60% of children exhibiting some response at 12 months preserved it after 2 years of treatment. Although the frequency of full response at 6 months (6.3%) in our monogenic SRNS cohort was lower than in nonmonogenic disease, partial response rate (21.3%) was comparable to nongenetic cases.[5,13] It can be postulated that drugs maintaining the integrity of the cytoskeleton in patients harboring mutations in certain genes might have a pronounced efficacy. For instance, in an animal model, WT1 knockout mice had reduced the expression of nephrin and podocalyxin.[14] Although a specific podocyte protein gene group was not shown to preferentially respond to CNI, due to the small number of patients in each category and the diversity and combination of types of variants in autosomal recessive traits, this possibility cannot be excluded. On the other hand, because of the lack of a comparator group, we cannot exclude that some patients might have experienced the rare natural course of spontaneous remission as previously reported in 6%

Table 4| Adjusted ORs (95% CI) for potential predictors of at least partial response to CNI at 6 months using stepwise logistic regression analysis

Characteristic	Full multivariable model (N = 121)	Model after backward elimination (N = 121)
Phenotype at presentation		
INS	[a]	
Overt NS	1.79 (0.36–8.86)	
CNS	ref.	
Biopsy		
FSGS	3.26 (0.62–17.00)	
DMS	4.72 (0.53–41.96)	
Other	0.91 (0.05–15.92)	
MCD	ref.	
Group of mutated genes		
Nuclear protein	0.52 (0.13–2.03)	
Actin cytoskeleton	0.88 (0.18–4.37)	
Mitochondrial	0.59 (0.10–3.52)	
GBM	0.68 (0.04–10.37)	
Other	0.39 (0.03–4.24)	
Slit diaphragm	ref.	
Biochemical parameters at CNI onset		
sAlbumin, g/l	1.16 (1.08–1.24)[b]	1.16 (1.08–1.24)[b]
Concomitant RAAS blockade		
Yes	1.18 (0.31–4.59)	
No	ref.	
Time of CNI onset from presentation		
>4.2 mo (50th centile)	1.36 (0.48–3.85)	
<4.2 mo (50th centile)	ref.	
CNI type		
Tacrolimus	0.46 (0.15–1.43)	
Ciclosporin	ref.	

CI, confidence interval; CNI, calcineurin inhibitor; CNS, congenital nephrotic syndrome; DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; INS, infantile nephrotic syndrome; MCD, minimal change disease; NS, nephrotic syndrome; OR, odds ratio; RAAS, renin-angiotensin-aldosterone system; ref., reference; sAlbumin, serum albumin.
[a]Not calculated due to the absence of patients with at least partial remission at 6 months within the CNS phenotype at the presentation group.
[b]$P < 0.001$.
Data are presented median (range).

Table 5 | Clinical and demographic characteristics of patients grouped by mutated gene

Characteristic	*WT1* variants (N = 15)	*NPHS2* variants (N = 49)	*NPHS1* variants (N = 15)	*P* value
Age at presentation, mo	25 (0–179)	24 (0–192)	28 (0–162)	>0.05
Female	13/15 (86.7)	28/49 (57.1)	10/15 (66.7)	>0.05
Presentation				>0.05
CNS	2/15 (12.5)	7/49 (14.3)	5/15 (33.3)	
INS	3/15 (20)	7/49 (14.3)	1/15 (6.7)	
Overt NS	10/15 (66.7)	35/49 (71.4)	9/15 (60)	
Biopsy				>0.05
MCD	4/15 (26.7)	13/49 (26.5)	5/15 (33.3)	
FSGS	6/15 (40)	26/49 (53.1)	6/15 (40)	
DMS	1/15 (6.7)	6/49 (12.2)	1/15 (6.7)	
Other	3/15 (20)	3/49 (6.1)	2/15 (13.3)	
Not done	1/15 (6.7)	1/49 (2)	1/15 (6.7)	
Response at 6 mo				>0.05
None	10/12 (83.3)	36/45 (80)	10/13 (76.9)	
Partial	2/12 (16.7)	8/45 (17.8)	1/13 (7.7)	
Full	0/11 (0)	1/45 (2.2)	2/13 (15.4)	
Kidney outcome				>0.05
Normal kidney function	6/15 (40)	13/48 (27.1)	7/15 (46.7)	
CKD stage 2–4	5/15 (33.3)	12/48 (25)	4/15 (26.7)	
Kidney failure	4/15 (26.7)	23/48 (47.9)	4/15 (26.7)	

CKD, chronic kidney disease; CNS, congenital nephrotic syndrome; DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis; INS, infantile nephrotic syndrome; MCD, minimal change disease; NS, nephrotic syndrome.
Data are presented as n/total (%) or median (range).

or fewer of primary focal segmental glomerulosclerosis cases irrespective of genetic background.[15] A few older publications have reported on spontaneous remission in CNS, yet lacking genetic analyses.[16] In the most recent years, this unexpected outcome has been described in case reports of patients with *NPHS1* and *TRPC6* variants.[17,18]

Kidney outcome

In the study by Mason *et al.*,[13] it was for the first time documented that irrespective of the pathophysiologic background (monogenic vs. nonmonogenic SRNS), response to immunosuppression is the key determinant of the long-term kidney outcome.

These findings are replicated in our cohort, as achievement of at least partial remission at 6 months after CNI initiation for all patients and for those with a follow-up of at least 2 years significantly attenuated the risk for kidney function decline by 75% and 65%, respectively. To our knowledge, this is the only study reporting on the kidney outcome of such number (N = 91) of CNI-treated monogenic SRNS children based on follow-up data of at least 2 years. However, longer-term data are required to draw definite conclusions on the role of CNI in kidney function preservation in monogenic SRNS.

Predictors of outcome

In our previous systematic literature review of CNI efficacy in monogenic SRNS, the identification of minimal change disease on the kidney biopsy and *WT1* variant carrier status were the only predictors of remission.[7] In this study, we did not identify any genetic associations predictive of response after CNI administration. Hence, we cannot propose that children with certain genotypes should be prioritized for such an intervention, yet the benefits from CNI treatment should be very carefully weighed against potential risks in specific patient groups. Children with *SMARCAL1* variants are at risk for overwhelming infections due to their inherent immune defect and those with certain WT1 variants are at risk for developing Wilms tumor (truncating and missense exonic variants) and gonadoblastoma (variants in intron 9 splice donor site).[19]

Notably, none of the patients with CNS remitted even partially. This observation is in line with the theory that in CNS, the underlying molecular defect is thought to impair glomerulogenesis in its very early stages.[20] In addition, higher serum albumin levels at CNI initiation were significantly associated with favorable response at 6 months. This observation could be explained by the relative abundance of plasma factors that affect the integrity of the slit diaphragm in patients with less severe nephrotic states, rendering its derangement more amenable to CNI.[21] Higher CNI levels were not consistently detected among patients with at least partial response compared with nonresponders; for instance, only tacrolimus but not ciclosporin C0 levels were higher among partially responsive patients at the 6-month evaluation, whereas the opposite was observed at the 12-month and last follow-up. Thus, the possibility that remission is more likely among children with "therapeutic" CNI levels cannot be excluded.

Strengths and limitations of the study

Compared with the previously published literature review by our group on the same topic, this study holds substantial advantages, first being the avoidance of publication bias.[7] Furthermore, complete and partial remission are defined in an objective way in accordance with the International Pediatric Nephrology Association clinical practice recommendations, incorporating serum albumin level, which is a more stable marker of disease activity not subject to diurnal variability.[2] Moreover, data on the use of RAAS blockers are available for all participants in order to allow formal analysis of their role in the induction of remission, in contrast to the important amount of missing information on their use in our previous publication.[7]

We do however recognize a number of limitations, first being the limited number of participants. Nevertheless, given the rarity of this diagnosis and the formal recommendation against CNI use in children with monogenic SRNS, it is unlikely that a larger number of patients can be recruited. Secondly, there are a small number of recruited patients from the American continent and of African descent. However, in the study by Sadowski *et al.*[1] only 2.6% of patients with monogenic SRNS with or without CNI treatment were of African ancestry. In the present

cohort of 141 patients with monogenic SRNS, there was only 1 subject of African ancestry as compared with the expected 4 cases. Of note, inclusion of a patient in the current cohort required treatment with CNI for at least 3 months.

Moreover, because of the retrospective nature of the study, there is a large heterogeneity in the adopted treatment strategies as well as in the systematic reporting of CNI side effects within medical records. Despite these differences, we have been able to demonstrate that response to treatment was not affected from delays in initiating CNI or drug blood levels and that no serious adverse events mandating drug discontinuation occurred (Table 2).

RAAS inhibitors were administered to over 80% of children. To our knowledge, there is no prospective study on the efficacy of antiproteinuric agents in monogenic SRNS. In the retrospective, multicenter study on the management of CNS by Dufek *et al.*,[22] an improvement in serum albumin was reported in some but not all patients after 4 weeks of treatment with RAAS blockade. However, these children still required weekly albumin infusions, and hence they were persistently nephrotic. Our patients with at least partial response to the CNI had a much stronger response with serum albumin levels of ≥30 g/l. In addition, the independent effect of RAAS blockade on response to CNI was not shown to be statistically significant in a logistic regression model (Table 4).

Finally, we have not collected data on long-term nephrotoxicity of CNI based on repeat kidney biopsy. Nephrotoxicity, not specifically confirmed on kidney biopsy, as a reason to discontinue therapy, was documented in 12 of 107 (11.2%) subjects. Thus, although there is a possibility that long-term CNI use could aggravate disease progression, we have shown benefit in kidney survival among patients who responded despite ongoing treatment.

CONCLUSIONS

On the basis of the results of this study, families of all children with SRNS could have a discussion with their pediatric nephrologist regarding the benefits and potential side effects of CNI treatment. An option would be to treat until the results from genetic testing become available and at that point evaluate response. Treatment should probably be discontinued if no response is attained after 6 months. The subgroup of patients with higher serum albumin levels could be the first candidates for such an intervention. On the contrary, treatment should be avoided in children with CNS, and its risks should be very carefully considered in patients with specific variants (*WT1* and *SMARCAL1*).

In summary, our data suggest that CNI therapy for monogenic SRNS might not be as futile as once believed to be. At least partial remission can be anticipated for up to 27.6% of children and mitigates the risk of kidney failure. In anticipation of a prospective randomized trial with longer-term data confirming our findings, a universal recommendation on CNI use in monogenic SRNS cannot be made at this point and instead the pediatric nephrologist and the patient's carers should decide jointly on a case-by-case basis.

APPENDIX

The CNI in Monogenic SRNS Study Investigators

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DISCLOSURE

KN has received consulting fees from Kyowa Kirin and Toa Eiyo Ltd. and payment for lecture from Chugai Pharmaceutical Co., Ltd. KK has received grants from Terumo Foundation for Life Sciences and Arts, Public Foundation of Vaccination Research Center, Taiju Life Social Welfare Foundation, Chugai Pharmaceutical Co. Ltd., Astellas Pharma Inc., Ono Pharmaceutical Co., Teijin Pharma Ltd., and Otsuka Pharmaceutical Co. Ltd., and payment for lectures from Tanabe Mitsubishi Pharma, Baxter Ltd., and Zenyaku Kogyo Co. Ltd. SF has received payment for lectures from Ferring Pharmaceuticals Co., Ltd., Asahi Kasei Pharma Co. Ltd., Teijin Pharma Co. Ltd., and Kyowa Kirin Co. Ltd. KI has received grants from Zenyaku Kogyo Co., Ltd., Daiichi Sankyo Co. Ltd., Enomoto Pharmaceutical Co., Ltd., Nihon Pharmaceutical Co., Ltd., Shionogi & Co., Ltd., Mochida Mochida Pharmaceutical Co., Ltd., JCR Pharmaceutical Co., Ltd., and Teijin Pharma Co. Ltd.; consulting fees from Zenyaku Kogyo Co., Ltd., Kyowa Kirin Co. Ltd., Sanofi K.K., Ono Pharmaceutical Co., Ltd., Teijin Pharma Co. Ltd., and Chugai Pharmaceutical Co., Ltd.; and payment for lectures from Novartis Pharma Co. Ltd., Shionogi & Co., Ltd., Zenyaku Kogyo Co., Ltd., Astellas Pharma Inc., and Chugai Pharmaceutical Co., Ltd.; and also pending patent for a therapeutic agent for Alport syndrome. GMo has received consulting fees from Bayer, Chiesi Farmaceutici, and Alnylam. MV has received grants paid to her institution from Italian Ministry of Health, Italian Medicinal Agency, European Society for Paediatric Nephrology (ESPN), and for sponsored studies from Alexion, Roche, Novartis, Bayer, Chinook, and Apellis; consulting fees from Novartis, Travere, Apellis, Roche, and Bayer; payment for lectures from Novartis; support for attending meetings from Novartis; payment for participation on a Data Safety Monitoring Board or Advisory Board from Apellis, Novartis, Achillion, and Biocryst; and also has taken an unpaid fiduciary role in National Kidney Foundation, European Reference Network for Kidney Diseases, ESPN, and Kidney Disease: Improving Global Outcomes. SL has received honoraria for lecture from Alnylam. MTPB has received payment for lecture to her institution by Kyowa Kirin. LTW has received grant from the Deutsche Forschungsgemeinschaft (DFG/German Research Foundation) on "Disease pathways in podocyte injury—from molecular mechanisms to individualized treatment options." All the other authors declared no competing interests.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Hee Gyung Kang, Dr. Seon HeeLim, and Dr. Howard Trachtman for the contribution of anonymized patient data in this study. They also thank the staff of the European Society of Pediatric Nephrology and International Pediatric Nephrology Association for disseminating the invitation for participation to the study to their members.

AUTHOR CONTRIBUTIONS

All authors have contributed to the conceptualization of the study, patient data acquisition, and interpretation of results. GMa wrote the first version of the paper. KT and DI reviewed the first version of the manuscript. All authors have revised and approved the last version of the manuscript before submission. GMa, DI, and KT had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Table S1. Patient country of origin and genotype.
Supplementary Table S2. List of variants detected in podocyte genes in participants of this study.
Supplementary Table S3. List of variants of unknown significance (VUS) in autosomal recessive genes in participants of this study.
Supplementary Table S4. Individual patient timeline.
Supplementary Table S5. Adjusted odds ratios (ORs) (95% confidence intervals [95% Cis]) for potential predictors of at least partial response to calcineurin inhibitors (CNI) at 6 months for patients with pathogenic allele combination effect using stepwise logistic regression analysis.
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