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ORIGINAL ARTICLE

Novel mutation patterns in children with steroid-resistant nephrotic syndrome

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ABSTRACT

Background. Idiopathic nephrotic syndrome (NS) in children poses treatment challenges, with a subset developing steroid-resistant nephrotic syndrome (SRNS). Genetic factors play a role, yet data on paediatric SRNS genetics in India are scarce. We conducted a prospective study using whole-exome sequencing to explore genetic variants and their clinical correlations.

Methods. A single-centre prospective study (October 2018–April 2023) enrolled children with SRNS, undergoing renal biopsy and genetic testing per institutional protocol. Clinical, histological, and genetic data were recorded. DNA isolation and next-generation sequencing were conducted for genetic analysis. Data collection included demographics, clinical parameters, and kidney biopsy findings. Syndromic features were evaluated, with second-line immunosuppressive therapy administered. Patient and renal outcomes are presented for patients with and without genetic variants.

Results. A total of 680 paediatric NS patients were analysed, with 121 (17.8%) having SRNS and 96 consent to genetic analysis. 69 (71.9%) had early SRNS, 27 (28.1%) late. Among participants, 62 (64.58%) had reportable genetic variants. The most common were in COL4A genes, with 20 (31.7%) positive. Renal biopsy showed focal segmental glomerulosclerosis in 31/42 (74%) with variants, 16/28 (57.1%) without variants. Second-line immunosuppressions varied, with CNIs the most common. Outcomes varied, with partial or complete remission achieved in some while others progressed to ESRD.

Conclusion. The study underscores the importance of genetic analysis in paediatric SRNS, revealing variants in 65.7% of cases. COL4A variants were predominant. Variants correlated with varied renal outcomes, highlighting potential prognostic implications. These findings emphasize the value of personalized approaches and further research in managing paediatric SRNS.

Keywords: COL4A mutations, genotype–phenotype correlations, paediatric nephrotic syndrome, SRNS, whole-exome sequencing

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KEY LEARNING POINTS

What was known:

- Paediatric steroid-resistant nephrotic syndrome (SRNS) presents therapeutic challenges, with genetic factors implicated in some cases. However, comprehensive genetic studies in the Indian population are lacking, necessitating further exploration into the genetic landscape of SRNS in this demographic.

This study adds:

- This study provides novel insights into the genetic basis of paediatric SRNS in India, identifying variants in 65.7% of cases. Col4A variants emerged as prominent, with implications for renal outcomes. The findings underscore the importance of genetic analysis in guiding personalized treatment strategies for SRNS.

Potential impact:

- By elucidating the genetic underpinnings of paediatric SRNS in the Indian population, this study lays the foundation for tailored therapeutic interventions and prognostic assessments. The identification of prevalent variants and their association with clinical outcomes holds promise for enhancing patient care and informing future research directions in the field of nephrology.

INTRODUCTION

Idiopathic nephrotic syndrome (NS) is the most common glomerular disease in children. Although most (85%–90%) respond to the steroid, 10%–15% remain non-responsive to the steroid during the initial presentation, and 14%–36% of patients become secondary non-responders during the course of the disease [1, 2]. Steroid-resistant nephrotic syndrome (SRNS) constitutes a therapeutically challenging and heterogeneous group of kidney disorders with persisting proteinuria, hypalbuminaemia, and oedema despite standard immunosuppressive therapy [3]. About 30%–50% of the SRNS patients progress to end-stage kidney diseases (ESKD) within 10 years [4, 5].

While the pathogenesis of SRNS is multifactorial, one-third of cases have a genetic basis involving mutations in various genes critical for podocyte structure and function [6, 7]. Advancements in genomic technologies, particularly whole-exome sequencing (WES), have provided unprecedented opportunities to explore the genetic landscape of rare and complex diseases, including SRNS [6]. WES allows for comprehensive analysis of the protein-coding regions of the genome, enabling the identification of potentially pathogenic variants and shedding light on the genetic underpinnings of SRNS in the paediatric population. Several genetic mutations and variants have been implicated in the pathogenesis of SRNS, affecting genes encoding key components of the glomerular filtration barrier, podocyte structure, and signalling pathways. Like most genetic disorders, hereditary SRNS shows ethnic and geographic differences [8]. The monogenic mutations associated with SRNS vary from different populations. Studies from the eastern and western parts of India did not show NPHS1 and NPHS2 gene mutation as common monogenic reasons for SRNS, as reported in the Western literature [9, 10]. There have been no large-scale genetic studies on paediatric patients with SRNS in the Indian population.

Moreover, a comprehensive understanding of the genotype–phenotype correlations in paediatric SRNS patients and the exact relationships between specific genetic variants and distinct clinical phenotypes, disease severities, and treatment responses have not been fully elucidated. Although expert opinion recommends against immunosuppression in patients with SRNS and proven genetic variants, anecdotal evidence suggests partial remission following treatment [11]. It is still uncertain whether second-line immunosuppression may retard the progression in these patients.

In this prospectively collected data, we aimed to identify and characterize genetic variants, including rare or novel ones, using WES, and examine their associations with various clinical phenotypes, disease severities, and compare renal outcomes of patients with genetic variants in comparison with those who did not have genetic variants.

MATERIALS AND METHODS

We conducted a single-centre, prospective, pragmatic study of children with SRNS from October 2018 to April 2023. As per the institutional protocol, all SRNS patients underwent renal biopsy and genetic testing for clinical care. For this study, clinical and kidney histological findings and genetic test information were recorded. Patients unwilling to undergo genetic testing were excluded from the study. The parents/guardians of the children signed informed consent forms for genetic studies. Each study participant was given sufficient information to make a fully informed decision on the need for genetic analysis. The privacy and confidentiality of the reports were maintained. The Institute Ethics Committee approved the study.

Definitions

SRNS was defined as failure to achieve complete remission after 6 weeks of treatment with 60 mg/m^2/day or a maximum of 60 mg/day oral prednisolone therapy according to the KDIGO (Kidney Disease: Improving Global Outcomes) clinical practice guidelines 2021 and the Indian Society of Paediatric Nephrology (ISPN, 2009) [12, 13]. It was ensured that patients who had been receiving corticosteroid therapy initiated outside of the institute by paediatricians had received adequate doses of corticosteroids or had been challenged with proper doses of corticosteroids before declaring SRNS. A response to treatment was defined as being complete remission (CR) if there had been three consecutive days of urine dipstick readings negative or trace, or a urine protein-to-creatinine ratio (UPCR) $\leq$0.2, and partial remission (PR) defined as a UPCR between 0.2 and 2 and a serum albumin concentration $\geq$ 3 g/dl. Steroid resistance was defined as 'lack of CR at 4 weeks of therapy with daily prednisone or prednisolone at standard doses' [13]. Early steroid resistance was defined as 'SRNS occurring in a patient during his first episode

of nephrotic syndrome', while late SRNS was defined as 'SRNS occurring in subsequent episodes' [14].

Genetic analysis

DNA isolation and next-generation sequencing were performed at the genetic laboratory approved for genetic testing. Four millilitres of whole blood were taken from patients and transferred into tubes containing 200 μl of EDTA for DNA isolation. DNA was isolated, and exome library preparation was performed using an Ion AmpliSeq™ Exome RDY Kit (Thermo Fisher Scientific, Inc.), which enabled high-efficiency enrichment, with >90% of the target bases covered at 20× and >90% uniformity. The target regions were amplified using the Ion AmpliSeq™ Exome RDY Library Preparation. Template-positive ISPs were enriched and sequenced on an Ion Proton™ according to the manufacturer's instructions. Ampliseq exome libraries were sequenced using the Ion Torrent platform and a Proton sequencer. Primary and secondary data analyses were performed with Torrent Suite v.5.0.5. The generated data were mapped to the GRCh37/hg19 genome sequence. Several final refinements, such as soft-clipping and adapter trimming, were routinely performed by the Torrent Suite on the server. Variant calling was performed by the inbuilt plugin Variant Caller vc 5.0–13. Annotation of this variant information was performed using Ion Reporter software.

All disease-causing variants reported in HGMD and ClinVar, as well as all variants with minor allele frequencies <0.05 in the gnomAD database, were considered. The investigation of relevant variants focused on coding exons and UTR regions. All potential modes of inheritance patterns are considered. *In silico*, nonsynonymous variants were predicted using multiple algorithms, such as PolyPhen-2, SIFT, MutationTaster, and Mutation Assessor. In addition, the provided family history and clinical information were used to evaluate identified variants concerning their pathogenicity and causality and were categorized into classes 1–5 according to ACMG guidelines. All variants related to the patient's phenotype, except benign or probably benign ones, were reported. Independent evaluation of the pathogenicity of the reported variants was performed by one co-author (K.M.) who specialized in medical genetics.

Data collection

All patients had their weight, height, body mass index, and blood pressure (BP) recorded, and nutritional status was graded according to IAP growth charts. Office BP was measured, and hypertension was defined as systolic and/or diastolic BP ≥ 95th percentile for age, sex, or height recorded on three or more occasions. The estimated glomerular filtration rate creatinine was calculated using the modified Schwartz formula [15]. Chronic kidney disease was staged according to the Kidney Disease Outcome Quality Initiative Guidelines [16]. All patients with SRNS were counselled for and, if consented, had undergone kidney biopsy after clinical stabilization and kidney tissue specimens were subjected to light microscopy, immunofluorescence (IF), and electron microscopy. Other supportive care was given accordingly, and the details related to the second-line immunosuppression drugs used and their duration were noted during OPD follow-up.

All the SRNS patients were examined for obvious syndromic features, such as deafness, pupillary abnormalities, absence of patella and abnormal nails, and obesity. A significant family history was sought. Those with correlating syndromic features with positive genetic tests or family history were given further second-line immunosuppression drugs as per treating clinician's discretion.

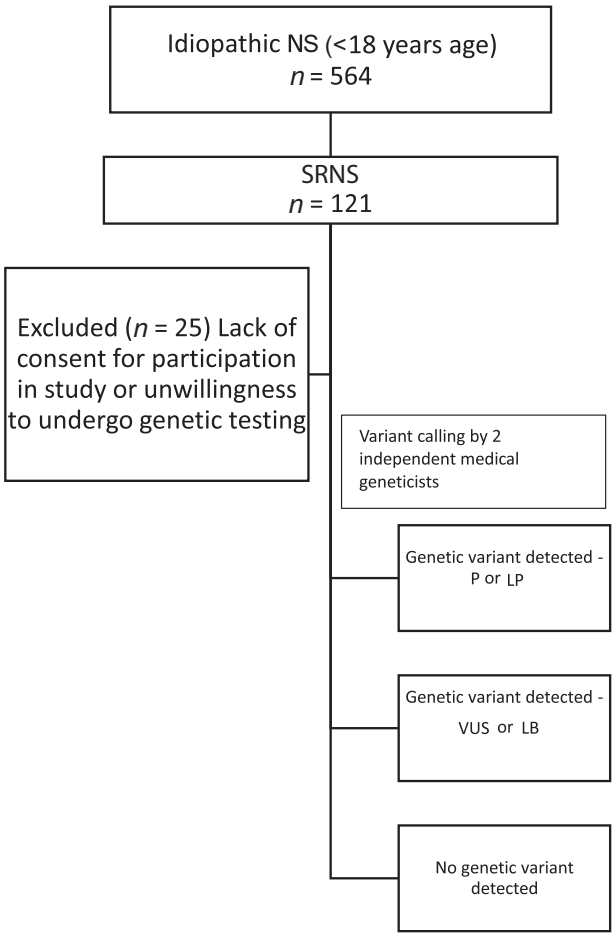

Figure 1: Study participants with SRNS.

Statistical analysis

Quantitative data are expressed as the mean ± standard deviation (SD). Categorical values are expressed as numbers and percentages. To analyse the significant differences between groups with or without pathogenic variants, categorical variables were analysed using the chi-square test or Fisher's exact test, and continuous variables were compared using the *t*-test or Mann–Whitney *U*-test. The statistical analysis was performed using SPSS v.25.0 (SPSS, Armonk, NY, USA).

RESULTS

A total of 680 paediatric NS patients aged between 1 and 18 years were analysed during the study period. Among them, 121 (17.8%) patients had SRNS and were advised to undergo genetic analysis; however, only 96 of them provided consent for genetic analysis. These 96 patients were included in the analysis. Among these patients, 69 (71.9%) had early SRNS, while 27 (28.1%) had late steroid resistance.

Among the 96 participants included in the study, reportable variants in single genes were present in 62 (64.58%) patients. After collation of data, the variants were reclassified as per ACMG

Table 1: Clinicopathological characteristics of patients in the study.

	Total ($n = 96$)	Genetic variants detected (P/LP) ($n = 23$)	Genetic variants detected (VUS/LB) ($n = 39$)	No genetic variants identified ($n = 34$)
Gender, male *n* (%)	69 (71.9)	18 (78.3)	27 (69.2)	24 (70.6)
Age in months, median (IQR)	132 (63.25–208)	201 (112–214)	116 (51–192)	127 (74–99)
Age of onset of NS, months median (IQR)	57.5 (26.5–158)	152 (63–194)	78 (23–145)	42 (22.5–101.5)
Hypertension *n* (%)	61 (63.5)	18 (78.3)	21 (53.8)	21 (61.8)
Early SRNS, *n* (%)	69 (71.9)	18 (78.3)	29 (74.4)	22 (64.7)
Late SRNS, *n* (%)	27 (28.1)	5 (21.7)	10 (25.6)	12 (35.3)
Parents consanguineous marriage	10 (10.4)	6 (26)	4 (10.3)	0
Family history	23 (24)	15 (65.2)	7 (17.9)	1 (2.9)
Syndromic features	18 (18.8)	13 (56.5)	4 (10.3)	0
Baseline eGFR, median (IQR)	83 (57–102)	54 (44–83)	87 (70–106)	84.5 (74–99)
Hb, g%, median (IQR)	11.5 (10.1–13)	10.7 (9.6–11.9)	11.7 (10.7–13.4)	11.3 (10.0–13.4)
Albumin, g% mean (SD)	2.7 (0.95)	3.1 (1.05)	2.6 (0.9)	2.2 (1.75–2.90)
Creatinine, mg%, median (IQR)	0.7 (0.5–1.7)	1.3 (0.7–3.0)	0.6 (0.4–1.3)	0.6 (0.5–1.5)
Triglycerides, mg% mean (SD)	200 (130–325)	147 (110–291)	213 (147–324)	227 (150–411)
Total cholesterol, mg% median (IQR)	236 (155–385)	164 (141–396)	246 (158–391)	309 (210–390)
Follow-up, months median (IQR)	27 (17–60)	27 (19–57)	20 (12–26)	44 (38.5–92.5)
ACEi/ARB use, *n*%	56 (58.3)	13 (56.5)	22 (56.4)	21 (61.8)

eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

2015 criteria [17]. An effort was made to determine how many variants can explain the disease. Among them, pathogenic (P) or likely pathogenic variant (LP) was detected in 23 (23.95%), while variants of uncertain significance (VUS) or likely benign (LB) were detected in 39 (40.62%) (Figure 1). A comparative analysis of various characteristics between participants with identified genetic variants (P or LP), identified genetic variants (VUS or LB) and those without any detected variant, including demographic, clinical, and laboratory parameters, is presented in Table 1.

Genetic variants

The genetic variants detected in the cohort

The details of patients with pathogenic and LP variants are detailed in (Table 2) while details of patients with VUS or LB are presented in (Supplementary Table S1). We encountered logistical constraints that prevented us from conducting genetic analyses on all the other family members. Still, all reports were analysed, and variant calling was done independently by two medical geneticists. We found a prevalence of autosomal dominant variants in genes, including INF2, LMX1B, CD2AP, PAX2, ACTN4, EHHADH, GAPVD1, SIX5, and COL4A3, ordered by frequency from highest to lowest. Autosomal recessive mutations, observed in descending order of frequency, were identified in genes such as *COL4A4*, *COL4A3*, *COQ8B*, *LAMB2*, *NPHS2*, *NPHS1*, *NUP160*, *PLCE1*, *BBS12*, *SGPL1*, *SMARCAL1*, *MYOE1*, *LAMA5*, *FAT1*, KIRREL1, *FAT1*, and *COQ2*. The X-linked mutations observed were COL4A5 and TBC1D8B.

Homozygous or hemizygous variants in COL4A3/4/5 genes ('The Alport sub-cohort')

The most common genetic variant detected in our cohort was in the COL4A genes. Twenty of the 62 (32.3%) with reportable variants were positive for COL4A-related genes (five in COL4A3, five in COL4A4, 10 in COL4A5). On variant calling, six had pathogenic, nine LP, and five VUS (Table 2 and Supplementary Table S1). The mean age in this sub-cohort was 10.05 (±5.8) years, and 15 (75%) were males, five (25%) had initial steroid responsiveness and developed late steroid resistance, 10 (50%) had developed other syndromic features suggestive of Alport syndrome during subsequent follow-up, while the remaining 50% did not develop any such features, which may be because of shorter follow-up of the cohort.

Renal biopsy in patients with steroid-resistant nephrotic syndrome

Renal biopsy was performed in 67 (69.2%) patients. Among them, FSGS was noted in 39 (40.6%) while MCD in 26 (27.1%). In patients with pathogenic or LP genetic variants, FSGS was seen in 13 (56.5%) and was more frequent than MCD, seen in 8.7% (Table 3).

Second-line immunosuppression use and response to calcineurin inhibitors and experience with specific therapy

Clinicians exercised discretion in administering second-line immunosuppressants to patients with SRNS. Notably, documentation accounted for patients who had previously received these agents before genetic testing or when they presented to our facility. Importantly, some patients received multiple immunosuppressive agents. The use of second-line immunosuppressive therapy is shown in Table 4 for all three groups.

Among the 23 patients with pathogenic or LP genetic variants, 10 had received further immunosuppression with calcineurin inhibitors (CNI), either cyclosporine or tacrolimus. Of these, two patients achieved CR and five patients achieved PR. The genetic variants associated with CR were NPHS1 and NPHS2, while those associated with PR were LMX1B, COL4A5, COL4A4,

Table 2: Genetic profile of patients with genetic variant classified as P or LP.

S. Number	Age at onset/ gender	Gene in which variant detected/ inheritance pattern	Clinical details (syndromic/non-syndromic) presentation	Type of mutation/zygosity	Variant description	Amino acid change	Pathogenecity	ACMG evidence of pathogenecity	Renal Biopsy	Outcome (patient/ renal)
1	9/M	BBS12/AR	Bardet Biedl Syndrome	Missense/ Homozygous	chr4:123663297G>A	p.Gly84Arg	LP	PM3, PP3	Not done	Alive/PR
2	17/F	LMX1B/AD	Nail Patella Syndrome	Missense/ Compound heterozygous	chr9:129455566, c.705G>C	p.Leu226Arg; p.Lys235Asn	LP	PP3, PM2		Alive/PR
3	6/M	COL4A4/AR	No	Frameshift/ Heterozygous	Exon 30/chr2:227920745	p.Pro878fs	LP	PVS1, PM		Alive/PR
4	16/M	COL4A3/AD	Yes	Splice site/ Heterozygous	splicesite_3:c.547–1delG		LP	PVS1, PM	FSGS	Alive/PR
5	17/M	COL4A5/XR	Yes	Nonsense/ Hemizygous	Exon 1,chrX:g108440189C>T, c.64C>T	p.Gln22Ter	LP	PVS1, PM	Not done	Alive/NR
6	17/M	COL4A5/XR	Yes	Frameshift deletion/ Hemizygous	Exon 36,chrX:10786989, c.3158delC	p.Pro1053LeufsTer99	LP	PVS1, PM		Alive/PR
7	13/M	COL4A5/XR	No	Missense/ Hemizygous	Exon 29, chrX:g.108606874G>A, c.2377G>A	p.Gly793Arg	LP	PP3, PM2	FSGS	Alive/PR
8	8/M	COL4A5/XR	No	Missense/ Hemizygous	Exon 40, ChrX:g.108667183G>C c.3604G>C	p.Gly1202Arg	LP	PP3, PM2	FSGS	Alive/ ESRD
9	6/F	COL4A4/AR	No	Missense/ Homozygous	Exon 45, Chr2:g227012226C>T c.4288G>A	p.Gly1430Arg	LP	PP3, PM2	FSGS	Alive/ ESRD
10	9/M	NPHS2	No	Missense/ Heterozygous	Exon 7, chr1:g.179552604C>T c.872G>A	p.Arg291Gln	LP	PM2, PM5	FSGS	Alive/CR
11	17/M	COL4A4	Yes	Missense/ compound heterozygous	Exon 39, chr2:227896932 c.3638G>A;	p.Gly1213Glu;	LP	PP3, PM2	Not done	Alive/PR
12	17/M	PAX2/AD	No	Missense/ Heterozygous	Exon 3, chr10: 102510464, c.226G>A	p.Gly76Ser	P	PS4, PP1	MCD	Alive/PR
13	17/F	LMX1B	No	Missense/ Heterozygous	Exon 4, chr9:129455529, c.668G>A	p.Arg223Gln	P	PS3, PP3	FSGS	Alive/PR
14	17/M	COL4A5/XR	Yes	Splice site/ Hemizygous	Exon 24, chrX:107840799		P	PVS1, PM	Not done	Alive/ ESRD

Table 2: Continued.

S. Number	Age at onset/ gender	Gene in which variant detected/ inheritance pattern	Clinical details (syndromic/non-syndromic) presentation	Type of mutation/zygosity	Variant description	Amino acid change	Pathogenecity	ACMG evidence of pathogenecity	Renal Biopsy	Outcome (patient/ renal)
15	4/M	COQ2	No	Frameshift/ Heterozygous	Exon 1, chr4:84206019, c.48dupC	p.Ala17fs	P	PS4, PVS	Not done	Alive/CR
16	16/M	COL4A3	Yes	Stop gain/ Homozygous	Exon 51, chr2:228175548, c.4812C>A	p.Cys1604	P	PM3, PVS	Not done	Alive/ ESRD
17	17/M	COL4A3	Yes	Stop gain/ Homozygous	Exon 51, chr2:228175548, c.4812C>A	p.Cys1604	P	PM3, PVS	Not done	Alive/ESRD (RTR)
18	2/F	NPHS1/AR	No	Missense/ Double heterozygous	Exon 9, chr19:36339610, c.1099C>T	p.Arg367Cys	P	PM3, PS3	Not done	Alive/CR
19	16/M	LMX1B/AD	Yes	Missense/ heterozygous	Exon 8 , chr9:129458594, c.1085A>G	p.Asp362Gly	LP	PM2, PP3	FSGS	Alive/ ESRD
20	8/M	COL4A5/XR	Yes	Frameshift deletion/ Hemizygous			P		MCD	Alive/PR
21	16/M	COL4A5/XR	Yes	Frameshift deletion/ Hemizygous			P		FSGS	Alive/ ESRD
22	12/M	COL4A5/XR	Yes	Frameshift deletion/ Hemizygous			P		FSGS	Alive/CR
23	1.5/F	COL4A5/XR	No	Missense/ hemizygous	Exon 38, ChrX:g.108457183G>C c.3604G>C		LP		MCD	Alive/PR

Table 3: Renal biopsy, immunosuppression use, renal, and patient outcomes of patients with SRNS.

Characteristics, *n* (%)	Total (*n* = 96)	Genetic variant detected (P/LP) (*n* = 23)	Genetic variant detected (VUS/LB) (*n* = 39)	No genetic variant identified (*n* = 34)	*P* value
Renal biopsy performed	67 (69.2)	15 (65.2)	25 (64.1)	27 (79.4)	
Renal biopsy pattern					
Minimal change disease	26 (27.1)	2 (8.7)	12 (30.8)	12 (35.3)	
FSGS	39 (40.6)	13 (56.5)	11 (28.2)	15 (44.1)	
Others	1 (1.04)	0	1 (2.6)	0	
Second-line immunosuppression					
Cyclosporine	16 (16.6)	4 (17.3)	5 (12.8)	7 (20.6)	
Tacrolimus	45 (46.9)	6 (26.1)	16 (41)	11 (67.6)	
MMF	11 (11.4)	0	6 (15.4)	5 (14.7)	
Cyclophosphamide	10 (10.4)	1 (4.3)	3 (7.6)	6 (17.6)	
Levamisole	10 (10.4)	1 (4.3)	4 (10.3)	5 (14.7)	
Rituximab	10 (10.4)	0	6 (15.4)	4 (11.8)	
Renal outcome at end of follow-up					
Partial remission	53 (55.2)	14 (60.9)	19 (48.7)	20 (58.8)	.565
Complete remission	17 (17.8)	3 (13)	11 (28.2)	3 (8.8)	.07
No response/ progressive renal failure	23 (24)	6 (26.1)	7 (17.9)	10 (29.4)	.668
Patient outcome at the end of follow-up					
Mortality	3 (3.1)	0	2 (5.1)	1 (2.9)	0.532

Table 4: Clinicopathological profile of patients with pathogenic/LP variant and response to calcineurin inhibitors.

S. Number	Age at onset/ gender	Genetic variant	Renal biopsy	Spot UPC[a] (g/g)	Serum albumin[a] (g/dl)	ACEi/ARB	CNI agent used	CR/PR	Time to first response (months)	Duration of CNI use (months)
1	1.5/M	COL4A4 (LP)	Not done	5.1	1.6	Yes	Tac	PR	4	24[a]
2	13/M	COL4A5 (LP)	FSGS	7.5	2.0	Yes	Tac	PR	3	13[a]
3	2/M	NPHS2 (LP)	FSGS	11	1.7	Yes	Tac	CR	2	24[a]
4	17/M	PAX2 (LP)	MCD	8.2	2.0	Yes	Cyc	PR	8	11[a]
5	17/F	LMX1B (P)	FSGS	4.7	2.6	No	Tac	PR	3	30[a]
6	2/F	NPHS1 (P)	Not done	3.8	2.7	Yes	Tac	CR	4.5	26[a]
7	12/M	COL4A5 (P)	FSGS	5.7	2.5	No	Tac	PR	3	12[a]

M, male; F, female; UPCR, urinary spot protein creatinine ratio; Tac, tacrolimus; Cyc, cyclosporine; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockade; CR, complete response; PR, partial response.
'duration' indicates the duration of CNI usage since the last follow-up.
[a]The ongoing usage of the CNI at the last follow-up.

and PAX2. Notably, except for two patients, all were on ACE inhibitors (ACEi) or angiotensin receptor blockers (ARB), and all were on low-dose steroids (Table 4).

Patient and renal outcomes

Of all the 96 patients, CR and PR were achieved in 55.2% and 17.8%, respectively, while 24% had no response and/or progressive renal failure. Among those with a pathogenic/LP variant, CR and PR were achieved in 13% and 60.9%, respectively, while 26.1% had no response (Table 3).

Three patients died at the end of the median follow-up of 27 months: two in the VUS/LB group and one in the 'no detected variant' group. The cause of death was due to infections in all three, and two of them had ESKD.

In the 'Alport sub-cohort', at the end of follow-up, five (25%) developed ESRD, one had no response, while one and 12 patients achieved CR and PR, respectively, with supportive care.

DISCUSSION

In this study, we observed a differing pattern of monogenic mutations responsible for the SRNS compared to the conventionally reported from Western literature. We observed that collagen gene mutation COL 4–3/4/5 mutations in a higher proportion of SRNS patients. The clinical presentation of COL 4 mutation as

the NS is uncommon. The gene encoding podocin, NPHS2, has been traditionally identified as a significant contributor to SRNS and is the most common monogenic cause of SRNS in European and North American cohorts [18]. Our study corroborated the infrequent occurrence of podocin gene involvement; only three out of 96 within our cohort had this genetic mutation, and in two of them, it was either pathogenic or LP. The observation was aligned with other Indian studies from the eastern part of India by Sinha *et al.*, where COL4A variants were identified as the predominant mutations leading to SRNS [10]. Our cohort found a higher prevalence of monogenic genetic variants (64.58%) in SRNS patients than that of 10%–35% reported in other studies [19, 20]. The reason for the higher prevalence may be mainly because of the selective referral of difficult-to-treat NS patients to this tertiary care institute. The other possible reason could be the higher incidence (16% of those with a genetic variant) of consanguineous marriage among the parents and founder mutation in the studied population.

Genetic mutations

The findings of our study shed light on the genetic landscape of paediatric patients with SRNS, providing valuable insights into the prevalence, spectrum, and clinical implications of genetic variants in this population. First, our study revealed a significant proportion of paediatric SRNS patients harbouring reportable genetic variants, with 64.56% of participants exhibiting one of such variants. This underscores the importance of genetic analysis in the diagnostic workup of SRNS, as genetic factors play a pivotal role in the pathogenesis of the disease [13]. Our findings are consistent with previous studies highlighting the genetic heterogeneity of SRNS and the diversity of underlying genetic mutations contributing to its aetiology [7, 21].

Immunosuppression

The KDIGO and IPNA guidelines [13, 22] recommend discontinuing immunosuppression for individuals with the monogenic form of SRNS. Nonetheless, a growing body of evidence suggests a positive response to CNI treatment, as observed in a study by Malakasioti *et al.* [23]. However, no specific mutation consistently demonstrated responsiveness to CNIs. In this context, a possible mechanism of CNI action is podocyte cytoskeletal stabilization [24]. CNI decreases IL-2 and IL-4, inhibiting the activation of a nuclear factor of an activated T cell (NFAT), a substrate of calcineurin (CN) in T cells [25]. Besides immunosuppressive effects, CNI has a direct effect on podocytes. CNI ameliorates podocyte injury by preventing the dephosphorylation of synaptopodin and stabilizes the podocyte cytoskeleton by upregulating the expression of cofilin, which was independent of its effect on synaptopodin [26].

Although genetic variant identification does not always indicate the stoppage of immunosuppressive therapy, one can expect a higher nonresponse rate in those with positive genetic variants. There is a need to balance the immunosuppression and the side effects of the drug. The varied genetic profile of the SRNS children in our cohort can pave the way for future research using other drugs for proteinuria reduction, and a widespread population-based study can further add to the knowledge regarding the same.

Further exploration of biomarkers indicative of CNI response in monogenic SRNS patients warrants comprehensive investigation. Considering that patients lack evident syndromic features or existing contraindications to these medications, a trial of CNIs might be considered feasible. Larger studies to address this question more comprehensively are needed to assess the potential utility of CNIs in this patient subset.

Outcomes

The Western and Indian literature reports a higher incidence of ESRD and nonresponse in patients with monogenic SRNS. We found that patients with monogenic and idiopathic forms of SRNS had similar outcomes. The possible reasons could be, first, due to the short duration of follow-ups of the present cohort; second, because of the differing frequency of genetic mutations from the Western studies; and third, our study sheds light on the fact that COL4A genes could be the most common forms of monogenic SRNS, as also reported from other Indian studies [9, 10]. It has been well identified that Alport kidney diseases need not always present with classic Alport syndrome(AS), and they may well with SRNS, and in this specific subgroup, renal failure may be slowly progressive to develop ESKD on longer follow-up; however, it is not universal [27, 28]. It is possible that many non-genetic forms of SRNS may respond to more prolonged treatment and may achieve remission [4, 5].

The most devastating diseases for patients and their families are those for which clinicians have no definite treatment available except for conservative care. After all, monogenic SRNS may not be a single disease, and an optimized trial of CNI combined with RAAS blockers may offer hope for at least some of these patients.

Strength and limitations

This study represents the largest single-centre investigation conducted in India, focusing on genetic variants identified in children with SRNS. The data collected prospectively underwent thorough examination by distinct medical geneticists, enhancing the reliability and robustness of variant calling. Nonetheless, our study is not without limitations, and we acknowledge that not all SRNS patients could be tested because logistics, consent for genetic testing, and financial constraints were factors in deciding whether to perform genetic testing and renal biopsy. The parents were not tested to determine the inheritance. This is unlikely to have influenced the results of this study because >80% of the patients had undergone genetic analysis. The pathogenicity of the variant observed could not be fully ascertained because the laboratory required samples from parents for genetic analysis. This could not be done due to cost limitations.

CONCLUSION

We present the most extensive genetic analysis data on SRNS in children, collected within a single centre. Our findings reveal a notable 64.56% incidence of monogenic causes, with 23.95% of cases exhibiting pathogenic or LP mutations. Specifically, our findings highlight the significant occurrence of COL4A variants leading to monogenic SRNS and unveil diverse phenotypic manifestations associated with these genetic mutations, which are not exclusively linked to Alport syndrome, and many such patients may develop clinical manifestations on extended follow-up.

Contrary to prevailing guidelines, our observations suggest the potential efficacy of a CNI in certain forms of monogenic SRNS, indicating promising clinical responses. However, larger-scale, multicentre studies with extended follow-up periods are essential to grasp these findings comprehensively.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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DATA AVAILABILITY STATEMENT

The data associated with the study is available with the corresponding authors. However, they cannot be made public because of ethical issues and institute policy.

CONFLICT OF INTEREST STATEMENT

None declared.

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